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I. DESTRUCTION OF SALVINIA THROUGH HERBICIDES*

By K. GEORGE

(*Received for publication on July 30, 1956*)

In West Bengal the need for controlling Salvinia has not so far been felt so acutely as in the case of some other weeds like water hyacinth (*Eichornia crassipes* Solms.) or water lettuce (*Pistia stratiotes* L.) owing to its rarer occurrence along with cultivation. In Ceylon, it is understood that Salvinia has become a menace to paddy cultivation and it is spreading to areas which have so far been free of infestation.[10] In the suburbs of Calcutta, the author has noticed *Salvinia natans* Hoffm. growing along with paddy only in some fields of Thakurpukur, Amtolla and Falta (in 24-Parganas District, i.e. 20 to 30 miles south of Calcutta). But *S. cucullata* Roxb. is seen growing abundantly in water-logged fields and jheels of the area between Sonarpur and Falta (about 25 to 30 miles south of Calcutta). *S. auriculata* Aubl. is found frequently in tanks and garden ponds in and around Calcutta. *S. cucullata* and *S. natans* are the indigenous species of Bengal while *S. auriculata* is introduced, being a native of Brazil and some other South American States. A study of the localities where it grows reveals that undrained muddy and sluggish waters of permanent pools containing organic debris favour the growth of this weed. It is found in greatest density during the rainy and winter seasons, although it may be present all the year round wherever it usually occurs. As the fields get dry in summer the species *S. natans* is seen to disappear almost completely but in some wet places it is found growing even in this season. All the members therefore are apparently perennial.

The above-mentioned three common species may be distinguished by the following characters (Table I; Fig. 1).

TABLE I

The distinguishing characters of the three species of Salvinia sporophyte commonly met with in West Bengal

	S. natans	*S. cucullata*	*S. auriculata*
Relative size of plant	Smallest	Medium	Largest
Size and shape of leaves	0·3″–0·5″ long, 0·15″–0·3″ broad, oblong with apex and base rounded. Sessile and horizontally spreading	Sessile. Upper rows of leaves conical or folded erect and crowded broader than long due to incurving, 0·5″–0·7″ wide	Upper two rows of leaves folded; each fold 1″ long and half as broad. Sessile erect or half erect
Nature of hairs and number	Upper side contains 12–24 pinnately arranged rows of papillose projections or infits of bristles. Underside of leaf thinly covered by short shining pellucid brown hairs	The upper surface is closely covered with very fine hairs and the under surface is nearly naked	Upper surface is covered densely with long velvety hairs which easily drip off any water falling from above; under surface by fragile brown hairs
Sporocarps number	2–8 in number in a cluster with regularly hexagonal wall cells	In bunches, more in number than *S. natans*	The clusters of sporocarps are in a scorpioid cyme
Shape	Each sporocarp spherical	Obovoid in shape	Elliptical in shape

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*The following herbicides were used in the present investigation: Cornox D (32% 2.4-D as an amine salt), Cornox M (32% M.C.P.A. as an amine salt), Agroxone 3 (27·5% M.C.P.A. as an active ingredient), Some other aspects of importance in the structure of the plant body in general are:

1. There are no proper roots, the submerged leaf being much dissected into filiform segments and thickly covered with hairs. The hairs, unlike root-hairs, are multicellular and rigid. The absence of proper roots suggests that the whole submerged surface is capable of absorption. The submerged lower row of leaves act as a drag against drifting and as a stabilizing organ of this floating weed.
2. The upper rows of leaves being densely covered with velvety hairs and entangling a large volume of air in them enable the plant to keep on floating by increasing the buoyancy.
3. The hairs prevent wilting of the leaves to a large extent in case of drought. They also help to drip away the water falling on the plants.
4. Vegetative method of multiplication is easily secured by fragmentation of the fragile stem.

Detailed descriptions of the structure of the plant body, the mode of its reproduction and propagation are given by several authors.[1, 2, 4, 8, 13] A survey of the literature reveals that no substantial study has been made regarding the effect of growth-regulating hormones on these plants. Parija (1934) used kerosene and copper sulphate to kill Salvinia.[11] His reports indicate kerosene used at the rate of 4 litres per 400 sq. ft. and copper sulphate of 0·006 per cent strength gave satisfactory killing of Salvinia. However, spray applications of these two chemicals in the above manner during the present investigations have shown that, although Salvinia was destroyed to a great extent, there was regeneration of the plants after certain periods. When spray applications with these materials were repeated after definite intervals the plants were apparently killed beyond revival.

Kar (1947) reported that 0·1 per cent solution of methoxone acted as lethal on this weed when sprayed on the leaves.[9] The present experiments with Agroxone 3 which is based on methoxone and three times as strong suggest that a higher concentration is necessary for complete lethal effect with one application of the spray (Table II).

It has been noted that it is difficult to recognize the plant for chemical application during its gametophytic life period and herbicidal treatments therefore could most advantageously be carried out when the young sporophytic plant has produced the first two pairs of leaves. That is, when the plant has attained a leaf size of not more than half a sq. cm. (Fig. 2).

Experiments.—Mature *S. auriculata* plants grown in large earthenware tubs were sprayed with different concentrations of the various herbicides, viz. Cornox D, Dicotox, Trioxone, Cornox M, Agroxone 3, C.I.P.C., Na.T.C.A., Karmex W, Weedicide, etc., to ascertain the minimum concentration of the chemicals required to destroy the plant within a period of one month. In a second series of experiments *S. auriculata* in their early stages of growth, when they had attained a leaf size of not more than half a sq. cm. and produced not more than three pairs of leaves, were sprayed with the above chemicals in a similar manner. The minimum herbicidal concentrations needed for complete killing in a month are indicated in Table II.

Crag Herbicide 1 (sodium 2.4-dichlorophenoxy ethyl sulphate), Ex. Herbicide 2 (wettable powder containing dichloral urea), Dicotox (10% 2.4-D as ethyl ester), Trioxone (2.4.5-T), Na.T.C.A. (sodium salt of trichloroacetic acid), Karmex W (80% C.M.U.), Weedicide (sodium arsenite), C.I.P.C. (70% chlorinated I.P.C. in xylene).

Observations on the order of death and decay.—Whenever the herbicidal concentrations of the growth regulator chemicals were sprayed no further growth of the plants could be observed immediately after application. The older upper leaves were found to be the first that got injured by the spray as they were expanded and floating on the surface of water and received the spray droplets in greatest quantity. The hairs were seen to entangle amidst them the spray droplets when they were applied in the form of suspension or emulsion and it may be concluded that the decrease of herbicidal effect in case of most of the other solution sprays was due to the fact that spray was dripped off by the velvety hairs. Whenever the hairs got a coating of the herbicidal spray they became dried up within four to six hours (Fig. 3). Either through the hairs or through the rest of the epidermal surface the herbicides were absorbed into the inner tissues of the leaves resulting in the destruction of the chlorophyll-bearing mesophyll tissues and transformation of the leaves to brown or chlorotic.

TABLE II

Comparative effectiveness of the herbicidal formulations at the two stages of growth of sporophytic Salvinia as expressed by the minimum percentage of the formulation required for lethal effect, when sprayed to give a complete coverage of the upper surface of the leaves

Stage of the plant	Cornox D	Dicotox	Cornox M	Agroxone 3	Trioxone	C.I. P.C.	Karmex W	Na. T.C.A.	Weedicide	Karmex in kerosene
Mature stage ..	5·0	3·5	6·0	5	3·5	2·5	2·0	12·5	3·0	0·5
Young or two-leaf stage ..	2·5	2·5	3·5	3	2·5	1·5	1·0	9·0	2·0	0·2

TABLE III

Duration of toxicity of various herbicidal formulations. Indicated by normal growth of two-leaf stage Salvinia in pots treated with 10 c.c. of 5% solution in each case. Expressed in number of days

Time of chemical treatment	Cornox D	Cornox M	Dicotox	Agroxone 3	C.I. P.C.	Karmex W	Na. T.C.A.	Trioxone	Weedicide	Crag Herbicide 1
August 1954	34	30	26	32	36	66	17	29	50	26
November 1954	42	40	29	40	38	74	19	32	58	26
February 1955	36	32	22	34	38	61	17	32	57	26

The wilting, drying and destruction of the hairs on the upper surface of leaves made the leaf more wettable and helped it to sink in the water. If the plants were killed with relative rapidity, that is to say, within a period of two days as in the case of C.I.P.C., sinking of the dead parts took place in about two weeks' time. While in the case of Karmex W which showed a slower lethal effect on Salvinia it took about three to four weeks for complete sinking of the dead plants. The maceration and decay of the killed plants were greatly facilitated by the residual herbicides themselves as well as by the fungal and algal growth on the dead plants. Colonies of the members of Cyanophyceae and Aspergillaceae were seen to thrive well on the decaying plants. It seems that during this time when the dead plants are floating it would be quite easy to collect and utilize them later for manurial purposes.

The decay of the upper leaves due to herbicidal effect was observed to continue to the nearest stem parts but translocation of the same towards the growing tip regions appeared to be extremely slow and the apical regions remained unaffected in case of many herbicides sprayed in low concentrations. It was evident that if translocation of herbicidal effect to the growing tips was not achieved within a period for which each sprayed chemical retained its toxicity revival of the plant was possible vegetatively. In the case of Karmex W the toxicity remained longer than any others (Table III) and it can reasonably be concluded that this slow detoxication is one of the main factors which make this chemical more efficient to bring about a complete lethal effect on Salvinia.

None of the symptoms such as proliferated growth of tissues, abnormal root or shoot initiation, epinasty or curling of leaves and stems, gall formation, abnormal cell elongation and other telemorfic modifications commonly shown by plants like bean, tomato, etc., treated with sublethal concentrations of 2.4-D, was evident in the case of Salvinia on treatment with any of the above formulations, excepting that plants which survived herbicidal application continued to grow much more slender and small as compared to the control plants. T.S. of stems of Salvinia killed by herbicides showed a darker brown colour of the vascular bundles especially the phloem region (Figs. 4 and 5), the lumen of these cells being filled with brown semi-solid matter. T.S. of the killed portions of leaves revealed that the cells are plasmolysed and collapsed and chloroplasts turned brown. After killing and before sinking the folds of the leaves were seen to spread out and lie flat on the surface of the water.

Retention of toxicity.—Retention of toxicity by the different herbicidal sprays was tested by putting fresh Salvinia plants in pots and spraying 10 c.c. of 5% solution. Whenever the plants were completely killed fresh young plants at the two-leaf stage were added to that place till a time when normal growth of it was possible in each pot. The time lapse required in each case was recorded. The same experiments were repeated two times more to minimize errors and average reading for the duration of toxicity shown by each chemical is marked in Table III.

Influence of rainfall after spraying.—Since the best absorption of the chemical was seen to take place through the hairs and upper surface of the leaves it was found highly essential that the sprayed material should remain on the surface for a sufficiently long time to ensure complete absorption. Whenever the rain fell immediately after spraying the chemical was washed off, making the effectiveness less intense. The minimum period for which the spray should remain on the sprayed surface undisturbed by the rain was studied by sprinkling water after definite intervals on the plants sprayed with herbicidal concentrations and draining away the water from the pot followed by replacing it with fresh water. In the case of Karmex W a minimum interval of two days was found to be essential for satisfactory herbicidal effect. All other chemicals used were seen to require shorter intervals and in the case of C.I.P.C. six hours' absorption was quite adequate.

Comparative effects of hormonal and non-hormonal herbicides.—Of the non-hormonal herbicides, kerosene, weedicide, and copper sulphate produced the most serious injury and killing of plant parts. A burning effect was apparent wherever they came in immediate contact, especially on the upper side of the floating and exposed leaves. In most instances they did not show a completely systemic herbicidal action as evidenced by their revival through new shoots from uninjured portions of the stem after definite intervals depending on the duration of toxicity retained by each material and its concentration. The failure of these contact herbicides to kill plants completely necessitated a second spray application at the time of regeneration in order to kill the plants beyond revival. An aqueous solution containing 1·5% Karmex W sprayed at such a rate as to give complete covering of the upper surface of the leaves took about three weeks for complete destruction of the plant in the early stages of its sporophytic growth with no survival of any part of the plant body. Addition of kerosene to Karmex W greatly enhanced its effectiveness and hastened the activity. 0·2 gm. of Karmex with 0·5 gm. of xylene or kerosene in a litre of water effectively killed the young sporophyte when sprayed at a rate sufficient to give a complete coating of the upper surface.

Recurrence of Salvinia through sexual reproduction.—In experimental pots as well as in small plots of fields wherein mature Salvinia were killed by herbicidal application, regeneration of the plants by sexual reproduction was noticed three to four months later. Many of the sporocarps produced by mature sporophyte well protected the sporangia inside from herbicidal action as well as from the unfavourable conditions like drought and heat of the summer. This probably gave rise to gametophytic life of the plant on the advent of the favourable rainy season. This leads to the conclusion that herbicidal destruction of sporophytic Salvinia after maturation and production of sporocarps may ensure no complete eradication. However, when herbicidal application was carried out in the early stage of the life-history of the sporophyte or during its gametophytic life period, recurrence of Salvinia was apparently little or nil thereby indicating that effective control of its occurrence in fields would be possible.

Field trials.—Small plots of fields in Thakurpukur area, a suburb of Calcutta where *S. natans* is seen to grow plentifully, were selected as site of the experiments. These plots of fields although water-logged during the whole of rainy and up to the middle of the winter seasons get completely dried towards the end of winter and during the whole of the summer seasons, i.e. from February to June. During the dry season Salvinia also is found to get dried away and almost completely disappeared. On the advent of monsoon rains in July the place gets filled with water again but growth of Salvinia sporophyte is apparently visible only by the month of September. This indicates that the plant passed its gametophytic life period during July to September in the accumulated water. When fields get excessively flooded the Salvinia also moves into the adjacent paddy fields, but continued growth of this weed along with paddy at a menacing intensity is seldom noted, possibly owing to the fact that these paddy fields do not remain water-logged for a prolonged period.

The plots thus selected were pits formed by the removal of the earth. In early August 1954, when there was little sporophytic Salvinia growing in these pits, herbicides were sprayed. Different concentrations ranging from 0·5 to 3% of Cornox D, Agroxone 3, Weedicide, C.I.P.C., Karmex W, Crag Herbicide 1 and Ex. Herbicide 2 were sprayed by means of 'Mysto' knapsack sprayers. A rate of 60 gallons per acre was employed to give a thorough coverage of the sprayed surface with the spray droplets. As all the pits were well bounded on all sides with soil barriers, there was no instance of sprayed herbicides flowing out during rains. This also prevented easy migration of fresh Salvinia from adjoining fields. In pits sprayed with minimum concentrations of 1·5 to 2% Agroxone 3, Dicotox, Weedicide and Crag Herbicide 1 and 1% Karmex W and C.I.P.C. were found to effectively prevent further occurrence of Salvinia till July 1955, while in the control pits there was growth of the sporophyte from late September to late December

1954. It may be noted that the sprayed chemical killed the Salvinia in the sprayed pits most probably during the gametophytic part of their life.

Of all the herbicides investigated, only Karmex W, Weedicide and C.I.P.C. killed Salvinia sporophyte at the early stages of growth in concentrations below 3% at the above rate of application. Whether these chemicals will have injurious effect on paddy was investigated by spraying on paddy the minimum herbicidal concentration of the above chemicals required for Salvinia. These experiments are carried out on both 'Aman' and 'Boro' paddy (*Oryza sativa*) grown in watery tanks in Bose Institute Experimental Farm at Shamnagar. The plants in both cases had grown to a height of no less than one foot. It was observed that concentrations as low as 0·5% aqueous preparation of both C.I.P.C. and Weedicide produced serious burning effect on contact with paddy. C.I.P.C. also turned the sprayed paddy plants chlorotic in the course of seven to ten days. While, spraying with 0·2 to 0·5% of Karmex-kerosene mixture showed the least toxic effect on mature paddy although paddy leaves looked greener in colour with localized dark spots for some days following spray application.

Discussion.—In Salvinia, the relative difficulty and slowness with which the herbicidal effect is translocated to the growing apical regions seems to be interesting. In this regard the behaviour of Salvinia is at variance from many of the land plants which on treatment with growth regulators like 2.4-D and M.C.P.A. showed more prominent injury first on the tender and growing regions at the top and later the injury continued to spread to the older portions below. Whether structural variation of the tissues of these two groups of plants is responsible for this different behaviour towards growth regulators is worthy of consideration.

T.S. of both stem and leaf of Salvinia shows a meagre development of the vascular bundles. In the stem the vascular tissues occupy a very small space in the centre, the rest of the space being filled with large parenchymatous tissue (Fig. 6). Day (1950–52) who made detailed studies on the absorption and translocation of 2.4-D in bean plant found the compound to traverse the cuticle, epidermis and mesophyll at a very small linear rate, i.e. around 30μ per hour, but once inside the phloem it moved at a much faster rate, from 10 to 100 cm. per hour.[6, 7] Also, Skoog (1938) showed that indole acetic acid having been absorbed by roots is mainly transported upwards in plants in the transpiration stream.[12] In view of the above findings it may be considered that as Salvinia is a water plant with very poor development of vascular tissues translocation of the chemicals from points of application to the other parts can take place only extremely slowly. Since the growing apex covered with two pairs of young folded leaves always remain below the water-level the sprayed materials in the normal course could not directly reach these portions but only by penetration from the spread out leaves floating on the surface of water which get destroyed first after receiving the spray in large quantity. It was noted by Crafts (1948) and Blackman (1950) that injury to mesophyll and phloem by stopping photosynthesis and plugging the sieve tubes would retard or stop downward translocation to other parts.[3, 5] The present experimental and observational evidence indicates that this is true.

In view of the poor translocation of the growth regulator herbicides, with the exception of 'Karmex W', they may also be considered in no way more effective than the non-hormonal herbicides in bringing about a complete killing. However, the herbicidal action of Karmex W on Salvinia, although slower than that of others initially, was evidently more systemic and complete, especially at the minimum concentrations of chemicals used.

With regard to the economics of chemical eradication of Salvinia, a herbicide which will destroy the weed with one spray application and produce the least crop-injury shall always be preferable; since repeated application for complete killing of the weed is likely to involve much greater cost by way of labour charges and cost of chemicals themselves. Experience from trials described above suggests that a spray application once of a 0·2 to 0·5% suspension of Karmex-kerosene mixture in water at the rate of 80 gallons per acre will destroy young Salvinia growing in paddy with minimized cost of application and crop-injury.

Employing the other hormone or non-hormone type of herbicides it may be suggested that destruction of Salvinia would be less costly and more easy at the gametophytic stage of growth which requires smaller quantities of herbicides. However, this method is possible only in fields where the sporophyte disappears completely in the hot season. If spray applications are to be carried out at a time when the field is free of the crop, higher concentrations like 5 to 10% of these herbicides sprayed at the rate of 20 to 30 gallons per acre by means of low volume spray equipments will probably bring about a more effective destruction of Salvinia. Stray occurrence of revived Salvinia owing to incomplete delivery of chemicals to all parts of the field is likely to necessitate a second spray application at the early stages of sporophytic growth wherever it is found.

Another suggestion of value in any eradication programme would be to conduct the herbicidal application in the largest area possible in order to minimize reinfestation by migration of the weed from nearby fields.

SUMMARY

The present experiments suggest the possibility of chemical eradication of Salvinia in paddy fields with minimized crop-injury by employing relatively low dosages of Karmex W. It may be best achieved by spray application of a Karmex-kerosene-aqueous mixture at the early stages of sporophytic growth of Salvinia. However, other types of herbicides may also be used if the destruction of the weed is undertaken during its gametophytic stage or when the field is free of the crop.

It is concluded that the failure of low dosages of 2.4-D type of hormonal herbicides to completely kill Salvinia is due to the poor translocation of the herbicidal molecules through the meagrely developed vascular tissue to the growing apical regions of the plant body. This consequently helps regeneration of the plant vegetatively soon after the toxicity of the sprayed chemical is over and it necessitates repeated herbicidal application for a complete destruction.

Some important precautionary measures to be adopted in a large-scale eradication programme are suggested.

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May & Baker (India) Ltd., Calcutta ..	Dicotox
Boots Pure Drug Co. (India), Ltd., Calcutta ..	Cornox D, and Cornox M
Imperial Chemical Industries (India) Ltd., Calcutta	Agroxone 3, Weedicide, and Trioxone

National Carbon Company (India), Ltd., Calcutta	Crag Herbicide 1, and Experimental Herbicide 2
E.I. du Pont de Nemours & Co. Inc., Delaware	Karmex W
U.S. Industrial Chemicals, New York ..	C.I.P.C.

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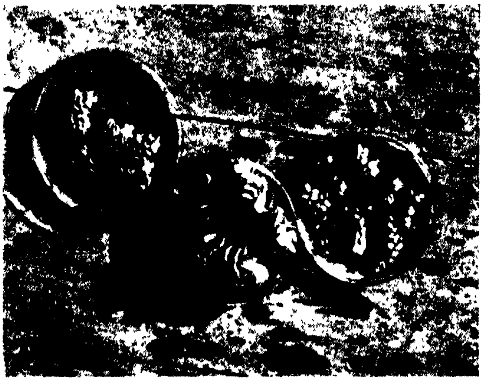


Fig. 1. The three species of Salvinia commonly seen in Calcutta and suburbs. (Left) *S. cucullata*, (Middle) *S. auriculata*, (Right) *S. natans*.

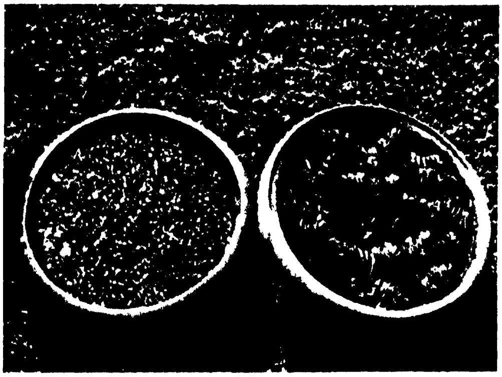


Fig. 2. The two stages of growth of sporophytic Salvinia. (Left) Early stage, (Right) Mature stage.

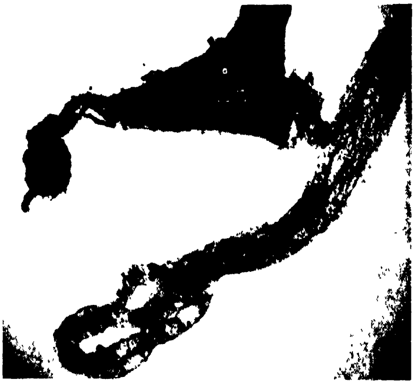


Fig. 3. Effect of herbicidal spray treatment on the velvety hairs of Salvinia. (Below) Control, (Above) A hair two hours after treatment with C.I.P.C.

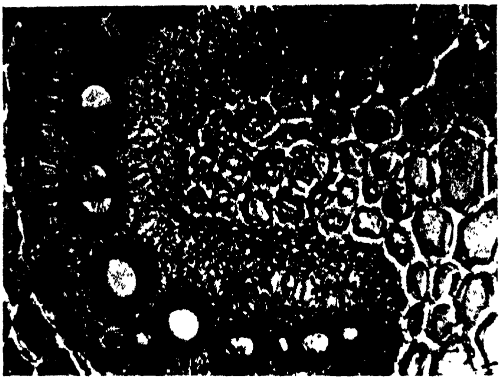


Fig. 4. T.S. of normal stem of *Salvinia auriculata*.

Fig. 5. T.S. of stem of Salvinia affected with herbicide.

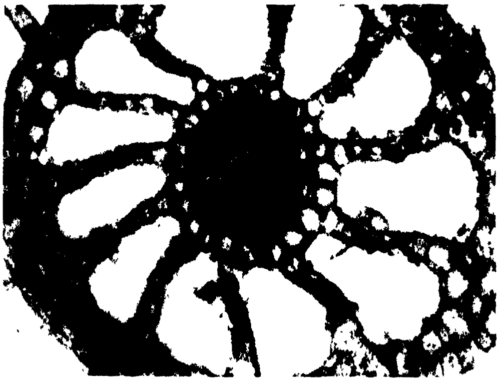


Fig. 6. T.S. of stem of Salvinia showing a meagre development of vascular tissue.

II. CHEMICAL CONTROL OF WEEDS IN THE PADDY FIELDS OF WEST BENGAL

by K. GEORGE

(*Received for publication on August 2, 1956*)

INTRODUCTION

Control or eradication of weeds in the crop fields of West Bengal by the hormone type of growth regulators has been the subject of detailed investigation at the Bose Research Institute for the last two years. Several selective herbicides chiefly derivatives of 2.4-D, M.C.P.A., 2.4.5.T. such as 'Dicotox', 'Cornox D', 'Fernoxone', 'Cornox M', 'Agroxone 3', 'Trioxone', etc., have been tried, but it was found that at concentrations necessary for controlling the weeds they were injurious to the crops also to a certain extent. In previous publications of the present author and others, the unique mode of action of 'Crag' Herbicide 1, as well as the successful control of weeds in banana, sugar-cane, field and sweet corn, asparagus, etc., without any injury to these crops, have been described.[1, 2, 3, 4, 6, 9, 10] As the main purpose of this paper is to give a reliable method of controlling weeds in paddy fields, no details of the experiments with some of the other weedicides giving unfavourable results are shown. The results described below confirm that (i) in accordance with earlier finding, 'Crag' Herbicide 1 acts as a germinating seed toxicant and (ii) it is active only in contact with soil and consequently does not affect established plants. Hence, this selectivity opens new possibilities in weed control programme. Further, data regarding yield as well as comparison of costs with conventional method of weeding are also given (Fig. 7).

Material and methods.—The experiments were conducted at the Experimental Farm of the Bose Research Institute at Shamnagar. The site of the experiment was a plot of land which was lying fallow for several years. Three adjacent plots of land of almost equal area (about 2,000 sq. ft. each) were prepared clean and free from all established weeds, in the manner that is usually adopted in paddy fields before sowing. Germinated 'Aus' paddy seedlings (*Oryza sativa*) were sown broadcast in the three plots of the field at a uniform rate, on the 24th June, 1954. The paddy was allowed to grow for a period of three weeks, to get established. The first plot was divided into three regions to try different concentrations of the chemical sprayed. The second plot was kept for conventional weeding and the third was the control plot. The first weed to appear in these paddy fields was *Croton bonplandianum* Bail. Although only a few in number, they had germinated almost simultaneously with the paddy and the emergence of this weed was so vigorous that in a period of three weeks it had attained a height of 2½ inches, which was almost equal to that of the paddy seedlings. At this stage, when the paddy had grown to a height of about 3 inches, spraying was carried out with aqueous solutions of 'Crag' Herbicide 1 in the three portions of the first plot on the 14th July, 1954. It had been observed in earlier trials[4] that a strength of 1 ounce per gallon of 'Crag' Herbicide 1 could safely control the germination of several weed seeds and therefore only three concentrations, viz. 0·5 oz., 1·0 oz. and 1·5 oz. per gallon, were tried. The spray was applied at the rate of 80 gallons per acre, using 'Smith Lightning' high volume sprayer. In other words, 2·5 lbs., 5·0 lbs., and 7·5 lbs. of the chemical per acre were applied. All the experimental plots were irrigated when necessary, but no manuring was done.

Observations and experimental results.—The continued growth of a few *C. bonplandianum* that germinated simultaneously and established along with paddy could not be controlled by the 'Crag' Herbicide treatment, but they did not grow to maturity subsequently, probably owing to the competition from paddy which grew much more vigorously later on. Any further germination of the seeds of other weeds, both narrow leaved grasses and sedges and broad leaved dicots, was prevented by an optimum concentration *of 1·0 oz. per gallon, i.e. a rate of 5 lbs. per acre of the herbicide (Figs. 1 to 3). 0·5 oz.* per gallon, i.e. a rate of 2·5 lbs. of chemical per acre, was found to be a little insufficient to control all the grasses, especially the perennial grass *Ischaemum rugosum* Salisb., popularly known as ' Marudi ' or ' Moraro '. This grass grew to maturity and produced fruits which ripened at the time of reaping the paddy. There was an initial set-back in growth of paddy seedlings for a few days following application of the spray. But this was more than made up by vigorous growth subsequently (Figs. 1 and 2). Conventional weeding was done twice in the second plot, first on 28th July and then on the 25th August. Still, continued growth of several weeds could not be completely checked (Fig. 5). Great difficulty was experienced in completely removing the grasses *Echinochloa colona* Link. (Jungle Rice) and *Ischaemum rugosum* Salisb., whose vegetative parts appear similar to that of paddy in the earlier stages of their growth.

In the third plot, kept as the control, the nature and growth of weeds and their succession, their density and competition with the crop were studied till the end of the experiments. As stated earlier the first weed to appear was *Croton bonplandianum.* But this was not so dense as the grasses that followed. There was profuse growth of these grasses (Fig. 4) and the one most dominant was *Echinochloa colona* Link. Numerically this was more than the total number of all other weeds. *Cyperus haspon* Linn. and *Ischaemum rugosum* were the next in the order of density. The other weeds of rarer occurrence were *Eclipta alba* Hassk. (Kessari, Kessuti), *Lippia nodiflora* Rich. (Bhuiokra), *Aeschynomene indica* Linn. (Kath sola), *Dichanthium annulatum* Staff., *D. caricosum* A. Cam., *Eleusine indica* Gaertn. (Crab grass), *Eragrostis tenella* R. and S. (Bharbhusi), *Cyperus defformis* L. (Barakerui), *Imperata arundinacea* Cyrill. (Ullu), *Fimbrystilis dichotoma* Vahl., *Rungia parviflora* Nees. var. *pectinata*, etc.

The paddy was harvested on 20th November, 1954, from each of the plots separately, from marked areas for purposes of comparison of the yield. On an average, the size of the ear from the herbicide treated paddy was bigger than either that of the hand weeded plot or the control plot (Fig. 6).

Data regarding cost and yield

Yield :

From control plot = 5½ mds. per acre.
From conventionally (hand) weeded plot = 14½ mds. per acre.
From herbicide treated plot = 20½ mds. per acre.
Increase in yield of herbicide treated plot over control plot = 250 per cent (about).
Increase in yield of herbicide treated plot over conventionally weeded plot = 43 per cent.

Cost of chemical weeding:

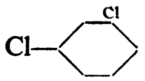
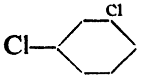
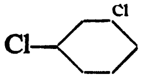
Rate of chemical application	= 5 lbs. per acre.
Cost of chemical @ Rs.8-13-0 per lb.	= Rs.44-1-0 per acre.
Number of persons engaged in spraying	= 4 per acre.
Spraying charges @ Re.1-12-0 per person	= Rs.7-0-0 per acre.
Total cost	= Rs.51-1-0 per acre.

Cost of conventional weeding:

Cost of weeding per acre in broadcast Aus paddy at the rate of Re.1-0-0 per person (Information supplied by State Agricultural Farm, Chinsurah)	= Rs.82-13-0.

DISCUSSION

The above data on yield, being the average of two readings from different regions of the experimental plots, are of a preliminary nature. Detailed investigations on the increase in yield after a randomized lay-out is intended to be undertaken in co-operation with the State Agricultural Farm at Chinsurah later. However, the above data gives a comparative idea of the costs of conventional and chemical weeding, the relative yields and the advantages of chemical weeding. As regards the cost of the conventional weeding a conservative estimate has been taken, but in actual practice the expenses will in all probability be more than that indicated here. The use of 'Crag' Herbicide 1 reduces the cost of weeding without the attendant hazards of other 2.4-D and M.C.P.A. formulations on sensitive crops (Figs. 1 and 2). With the increase in the soil temperature in the humid month of July, there had been a uniform germination of many weed seeds and spray applied, just after this total emergence, gave good control of weeds (Fig. 1 Treated; Fig. 4 Control).

As mentioned above, there was an initial set-back or stunting of the growth of paddy seedlings after the Crag Herbicide treatment. This observation leads to the conclusion that phyto-toxic materials are formed as a result of soil activation of 2-(2.4-dichlorophenoxy) ethyl sulphate  —O—CH$_2$CH$_2$—O—SO$_3$Na, which would otherwise have been inactive. This herbicidal activity of 'Crag' Herbicide 1, previously referred to as soil activation, is in all probability now specifically attributed to activation by micro-organisms like *Bacillus cereus* var. *mycoides* (Flugge) Smith, Gordon and Clark.[1, 9, 10] Further, it is also known[1, 10] that the herbicide undergoes a chemical change involving either acid or enzymatic hydrolysis to 2-(2.4-dichlorophenoxy) ethanol  —O—CH$_2$CH$_2$—OH followed by the biological oxidation of the latter compound to 2.4-dichlorophenoxy acetic acid  —O—CH$_2$COOH. Both these products thus formed are highly active plant growth regulators and the paddy roots on the surface of the soil may have absorbed a portion of these substances thus causing an initial set-back in the plants' growth. However, the amount of these reactive end-products with hormone-like effect would be extremely small and therefore easily tolerated or assimilated by the already established crop plants and it appears that they are further converted down to new products which could act as growth promoting hormone, in view of the greater subsequent growth of the paddy.

Tottingham (1918)[8] others[5,7] have suggested that addition of sulphur to the soil promotes the growth of the plants by increasing the protein synthesis. If so, the increased vegetative activity recorded earlier may also be explained on the basis of the formation of sulphates by the breakdown of sodium 2.4-dichlorophenoxy ethyl sulphate on activation by soil micro-organisms. Further investigations will be necessary to throw more light on the mechanism of the reaction resulting in the formation of growth promoters from plant growth regulators.

SUMMARY

With an optimum dosage of 5 lbs. per acre of 'Crag' Herbicide 1, control of most of the annual weeds in paddy fields was possible, without the serious crop-injury that is usually associated with other 2.4-D. and M.C.P.A. formulations.

At a later stage in the life of the 'Crag' Herbicide treated paddy, it was observed that there was increased vegetative growth and it is concluded that some growth promoting substances are formed during the biological activation processes undergone by this growth regulator, but the confirmation of this requires further investigation.

ACKNOWLEDGEMENT

The author wishes to thank the National Carbon Company (India) Ltd. for free supply of 'Crag' Herbicide 1, Dr. D. M. Bose, Director, Bose Institute, for giving necessary facilities, and Dr. K. T. Jacob, Head of the Department of Botany, for giving guidance and criticism during the course of these investigations.

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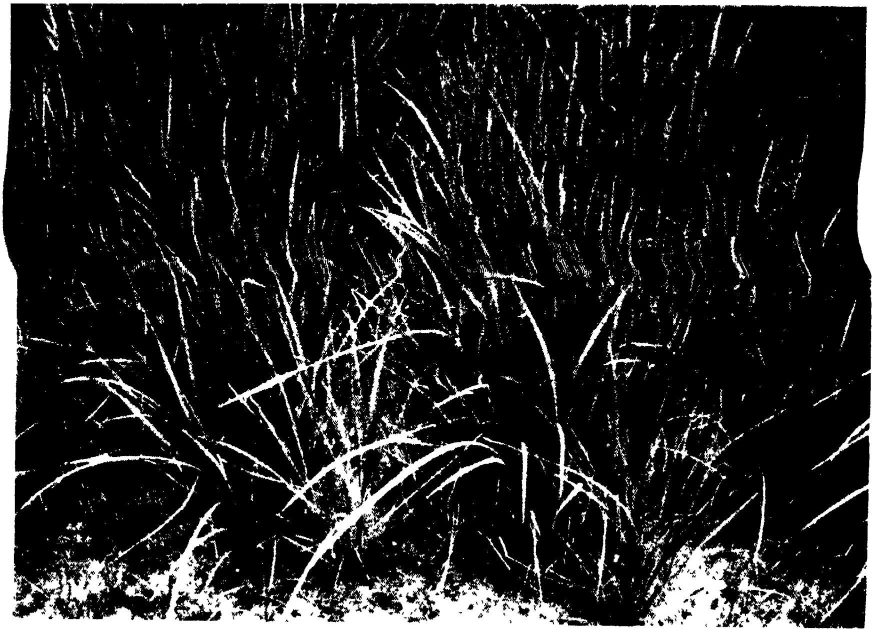

FIG. 1 Paddy treated with 'Crag' Herbicide I, at the rate of 5 lbs per acre. 55 days after spraying.

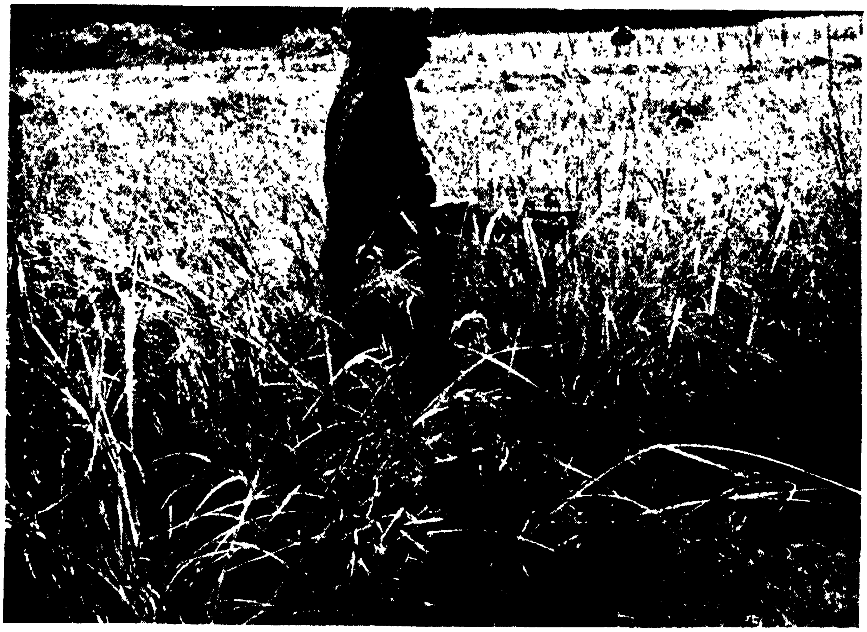

FIG. 2. Herbicide treated paddy at the time of reaping.

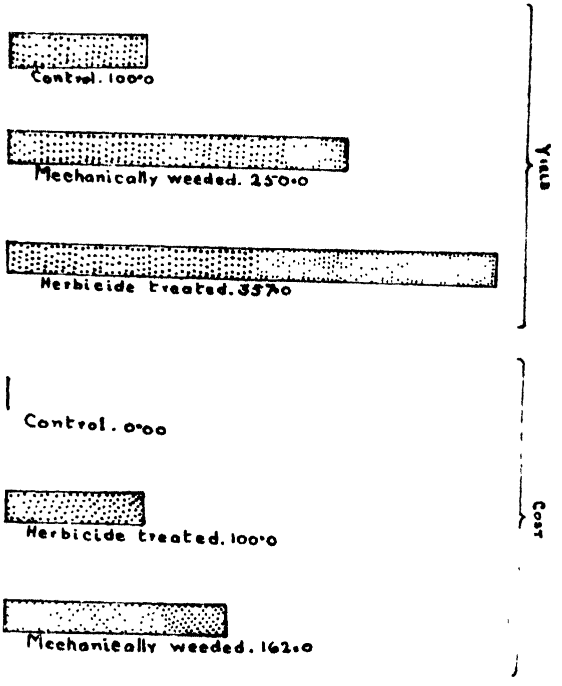

FIG. 7. Data on costs and yield in the different methods of weeding. Read 'Manually' instead of 'Mechanically'.

III. PAPER CHROMATOGRAPHIC SEPARATION OF BERBERINE AND SOME OF THE RELATED ALKALOIDS

By D. P. CHAKRABORTY

(*Received for publication on December 22, 1956*)

Paper chromatographic studies of plant constituents have been extensively pursued, specially for their identification, or determination of their homogeneity. The application of this method to alkaloids has been investigated by various workers and reviewed by Romeike.[1] In view of the occurrence of berberine and related alkaloids in a large number of plant families,[2] it was of interest to undertake the present investigation. Erbling and Wulf[3] experienced difficulties in determining the R_f value of berberine, using aqueous alcohol as the solvent. In the present communication, methods have been worked out for separation and identification of the alkaloids, berberine, tetrahydropalmatine, tetrahydrojatrorrhizine, tetrahydrocoptisine, neprotine hydrochloride, and himanthine.

The R_f values have been determined using several solvent systems by the unidimensional ascending technique. Experiments have also been carried out on papers treated with buffer solutions of different *p*H.

The names of alkaloids and their sources are given in Table I. At the foot of Table II the solvents are listed.

TABLE I

	Name	*Source*[5]
1.	Berberine	*Berberis vulgaris* L.
2.	Tetrahydropalmatine	
3.	Tetrahydrojatrorrhizine	
4.	Tetrahydrocoptisine	*Coptis teeta* Wall.[6]
5.	Neprotine hydrochloride	*Mahonia nepaulensis* D.C.[7]
6.	Himanthine	*Berberis himalaica* Ahr.[8]

EXPERIMENTAL

Saturated ethanolic solutions of the specimens were prepared at 25°C. Approximately 10 μl. of the solutions were applied to Whatman no. 1 filter paper, the methods and procedure being the same as described by Burma and Banerjee.[9] Treated papers were prepared using buffer solutions of different *p*H by the usual method of ascending chromatography. This ensured uniform impregnation of the buffer in the paper. The buffer solutions containing 0·1 *M* citric acid and 0·2 *M* sodium hydrogen phosphate were prepared by the methods detailed by Clark.[10] The papers were air-dried before use. The solvents were of the purest quality available. In the case of mixed solvents, the upper phase, in general, was used except where otherwise specified. Treated papers were prepared using the buffer solutions of *p*H 4·8, 6·8 and 8. Of these the results obtained at *p*H 4·8 and 6·8 are given in Table II.

TABLE II

R_f values of berberine and some related alkaloids with different solvents

	Solvents																				
Alkaloids	1a	1b	1c	2a	2b	2c	3	4a	4b	4c	5	6a	6b	6c	7	8	9	10	11	12	13	14
Berberine	·04	·12	·04	·51	·60	·66	·28	·29	·22	·27	·12	·23	·35	·36	·27	·36	·37	·09	·16	·12	·29	·04
Tetrahydropalmatine ..	—	·37	—	·85	·86	·95	·95	·90	·76	·95	·91	·71	·91	·93	·68	·74	·80	·49	·59 ·11	·47 ·11 ·35	·94	*
Tetrahydrojatrorrhizine ..	·25	·42	0	·73	·73	·90	·92	·89	·66	·90	·88	·64	·88	·88	·69	·73	·72	·49	·53	·46	·90	·34
Tetrahydrocoptisine ..	—	—	—	—	·70	·96	·10	·90	·83	·96	·96	·72	—	·92	—	—	—	—	—	—	—	—
Neprotine hydrochloride..	0	·12	·11	·53	·55	·67	·11	·26	·24	·23	·10	·29	·38	·44	·29	·36	·40	·20	·20	·35 ·15	·40	·08
Himanthine	0	·29	0	·60	·49	·66	·99	·90	·14	·24	†	·20	·85	·95	·81	·83	·82	·07	·46	0	·40	0

* Long trailing † Solvent front

1. (*a*) Water (22·5°C.); (*b*) water with formic acid at *p*H 4·8 (22°C.); (*c*) water with ammonia at *p*H 9·4 (22°C.).
2. (*a*) *n*-Butanol : acetic acid : water (25 : 2 : *V*) where *V* is the vol. required to saturate the mixture (23·5°C.); (*b*) *n*-butanol : acetic acid : water (25 : 2 : *V*) on treated paper at *p*H 4·8 (23·5°C.); (*c*) *n*-butanol : acetic acid : water (25 : 2 : *V*) on treated paper at *p*H 6·8 (23·5°C.).
3. *n*-Butanol : ammonia (sp. gr. 0·91) : water (25 : 3 : *V*) (25·5°C.).
4. (*a*) *n*-Butanol saturated with water (29°C.); (*b*) *n*-butanol saturated with water on treated paper at *p*H 4·8 (29·5°C.); (*c*) *n*-butanol saturated with water on treated paper at *p*H 6·8 (30°C.).
5. *iso*Propanol : ammonia (sp. gr. 0·91) : water (10 : 1 : 1) (25°C.).
6. (*a*) *iso*Propanol : water (10 : 2) (29·5°C.); (*b*) *iso*Propanol : water on treated paper at *p*H 4·8 (25°C.); (*c*) *iso*Propanol: water on treated paper at *p*H 6·8 (30°C.).
7. Formic acid (85%) : water (1 : 9) (22°C.).
8. Acetic acid : water (1 : 9) (22°C.).
9. Propionic acid : water (1 : 9) (20°C.).
10. Pyridine: water (1 : 9) (25°C.).
11. Formamide: water with formic acid at *p*H 4·4 (1 : 9) (23·5°C.).
12. Water saturated with collidine (lower phase) (30°C.).
13. Collidine saturated with water (upper phase) (30°C.).
14. Ethylene glycol. : water (9 : 1) (25·5°C.).

All the experiments were carried out at room temperature ranging from 20° to 30°C. The temperature for a particular experiment has been stated in parentheses along with the solvent system used.

Spots were detected by observing the fluorescence of the compounds in ultra-violet light (Chromatolite, 2537 Å), as well as from the colour developed by spray reagents, which included Dragendorff's reagent as modified by Munier and Macheboeuf.[11]

RESULTS

The R_f values of the alkaloids in various solvent systems are recorded in Table II. The nature of colour or fluorescence given by the alkaloidal spots is recorded in Table III.

TABLE III

Colour produced by different chromogenic spray

Y	Yellow	Y-bn	Yellowish-brown
Gy	Greenish-yellow	Bn	Brown
Br	Brick red	Py	Pale yellow
Gr	Garnet red	Dy	Dull yellow

	In ordinary light	NH_3-vapour	2*N*-NaOH	In U-V light	In U-V light (+NH_3 vap.)	Dragendorff's reagent
1. Berberine	Y	Y	Y	Gy	Gy	Y-bn
2. Tetrahydro-palmatine	—	—	—	Y	Dy	Bn
3. Tetrahydro-jatrorrhizine	—	—	—	Dy	Dy	Bn
4. Tetrahydro-coptisine	—	—	—	Br	Br	Bn
5. Neprotine hydrochloride	Y	Gr	Gr	Y	Gr	Y-bn
6. Himanthine	—	—	—	Py	Y	Bn

The spots were usually elongated in character. The nature of the elongation was found to vary with different alkaloids. Variation of the size and shape of the spots has been observed with different solvent systems as well as with different types of paper used.

Himanthine gave a round spot in water or in neutral solvents. The spot elongation was minimized when papers treated with buffer solutions of *p*H 4·8 were used. The dragging effect increased with increasing *p*H of the buffer solution and at *p*H 8 the spots were very much elongated in character. The movement of the compounds was also faster than that at lower *p*H. With ethylene glycol, the trailing effect was very marked. The use of water as the solvent gave valuable indications as to the nature of the bases.

Attempts were also made to separate the constituents from a mixture of all of them. The results show that, with the help of a suitable pair of solvent systems, it may be possible to separate all the six components by the two-dimensional technique.

DISCUSSIONS

It is apparent from Table II that R_f values of the compounds increase with the addition of organic solvents to water. Change in R_f values has also been observed with water at different *p*H. In acidic *p*H, spots are less elongated and more well defined due to suppression of dissociation.

The spot shape of alkaloids with respect to their dissociation constants or *pk* values has been discussed by Munier.[4] It has been shown by him that the shape of spots might be less elongated if the numerical value of *p*H is near about that of the *pk* of the alkaloids. Dutta[12] determined the apparent *pk* values of berberine and neprotine hydrochloride by spectrophotometric method and found them to be 5·86 and 5·81 respectively.

The spots of these substances are elongated in neutral or alkaline medium but in acidic medium the elongation is not so marked. The results are consistent with the findings of Munier.[4]

Himanthine has been observed to give round spots in neutral solvents. It is likely that the compound has a dissociation constant outside the range specified by Munier as it belongs to bis-*iso*quinoline series.

The R_f value of a particular alkaloid of the series has been found to increase with a decrease in *pk* of the acidic solvent used. Thus the R_f values of the alkaloids studied here are less in formic acid than in acetic or propionic acid. The R_f values of the alkaloids in acetic or propionic acid differ little. These two solvents have nearly the same *pk* values.

It appears from the present investigation that the R_f values in conjunction with the fluorescence behaviour may offer a valuable clue into the identity of these alkaloids. The most suitable solvents for the purpose have been found to be the following:

(*a*) *n*-Butanol : acetic acid : water (25 : 2 : *V*) on treated paper at *p*H 4·8; where *V* is the vol. required to saturate the mixture;
(*b*) *n*-Butanol saturated with water;
(*c*) *n*-Butanol saturated with water on treated paper at *p*H 4·8;
(*d*) Formamide : water with formic acid at *p*H 4·4 (1 : 9);
(*e*) Collidine saturated with water (upper phase).

With alkaline solvents, the spots of neprotine hydrochloride were garnet red in colour during development. But the colour soon turned yellow on exposure to air. This is due to the fact that at first the garnet red base is liberated[5] and this is readily oxidized by air to the yellow compound. A close proximity of R_f values in the different solvents has been observed in the case of berberine and neprotine hydrochloride which are similar in structure.[13] Tetrahydropalmatine, tetrahydrojatrorrhizine and tetrahydrocoptisine show a similar behaviour. While the R_f values of himanthine in some solvents are nearer to those of berberine and neprotine hydrochloride, in other solvents they approach those of tetrahydropalmatine and tetrahydrojatrorrhizine.

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IV. TRITERPENOIDS.

PART V. TRITERPENES FROM *LUFFA CYLINDRICA* ROEM.

By A. K. Barua *and* P. K. Bose

(*Received for publication on March 3, 1957*)

This climber, belonging to the family *Cucurbitaceae*, is widely distributed in India. In Bengal it is known as *dhundul.* The green fruit is edible and is used in curries, etc., and the dry mature fruit, which has an interwoven network of fibre, is used as a skin brush. The seeds are said to be emetic and cathartic.

Peakolt[1] reported the presence of saponin in the fruits of *L. cylindrica* Roem. From the seeds, de la Torre and Santos[2] isolated a bitter substance, m.p. 182–184°, and Mendoza, Cruz and Santos[3] obtained a bitter saponin, m.p. 268–270°, which on acid hydrolysis furnished a genin, m.p. 283–285°. The saponin was found to be highly toxic. Rangaswami and Sambamurthy[4] also reported the isolation of a bitter principle of m.p. 182–184° from the seeds of *L. aegyptica* Mill. (syn. *L. cylindrica* Roem.). They also obtained evidence for the presence of saponin in the seeds.

In the course of our survey of saponin-bearing plants of India, we re-examined the seeds of *L. cylindrica* Roem. (bitter variety). The seeds were collected from local market.

The dried powdered seeds were extracted successively with petroleum ether (b.p. 60–80°), ether, benzene, chloroform and alcohol (95%). From the alcoholic extract a crystalline saponin was isolated in good yield. It becomes slightly brown at 265° and sharply decomposes at 271°. The crude saponin, on hydrolysis with alcoholic hydrochloric acid, gives an acid sapogenin in good yield and a neutral sapogenin in extremely poor yield.

The acid sapogenin has the molecular formula $C_{30}H_{48}O_3$, m.p. 304–307°; $[\alpha]_D^{24}+$ 78·3° ($CHCl_3$) and gives a pale yellow coloration with tetranitromethane. It gives a monoacetate, $C_{32}H_{50}O_4$, m.p. 259–261°; $[\alpha]_D^{24}+72{\cdot}2°$ ($CHCl_3$) and a monomethyl ester, $C_{31}H_{50}O_3$, m.p. 198–200°. The methyl ester gives a monoacetate, $C_{33}H_{52}O_4$, m.p. 220–222°.

All the physical and chemical properties of the acid agree with those of oleanolic acid and its identity has been confirmed by comparison of mixed m.p. of the methyl ester with an authentic sample of methyl oleanolate.

The neutral sapogenin fraction, which was obtained in extremely poor yield, was chromatographed over Brockmann alumina and was repeatedly crystallized from aqueous ethanol until a constant melting point of 176-177° was reached. It appears to have the molecular formula, $C_{30}H_{48-50}O_3$. It responds to Liebermann-Burchardt reaction and gives a pale yellow coloration with tetranitromethane.

Due to the poor yield of the substance, further work could not be carried out and attempts are being made to isolate the product in larger quantities.

Experimental

(The m.p.'s are uncorrected. The micro-analysis of the samples has been carried out by Mrs. C. Dutta.)

Isolation of the Saponin

Dry powdered seeds (1·5 kg.) were exhaustively extracted successively with petroleum ether (b.p. 60–80°), benzene, chloroform and alcohol (95%), in a Soxhlet extractor. The volume of the alcoholic extract was reduced to about 1 lit. and was left overnight when large quantity of an almost white solid material separated. It was filtered, washed with alcohol and dried. Yield 14·5 gm. It gives the tests for saponin.

The crude saponin (4·5 gm.) was dissolved in 50 c.c. of distilled water and 500 c.c. of alcohol was added to it when fine colourless crystals immediately separated. It was crystallized six times in the same manner and was dried at 140° in vacuum over P_2O_5 for 10 hours. It is almost insoluble in all organic solvents but highly soluble in water. When heated, it slowly becomes brown at 265° and sharply decomposes at 271°. When burnt, it leaves a very small quantity of ash which has been found to contain potassium.

Hydrolysis of the Crude Saponin: Isolation of Oleanolic Acid

The crude saponin (10 gm.) was dissolved in water (250 c.c.) and 1200 c.c. of alcohol (95%) and 310 c.c. of conc. hydrochloric acid were added. The mixture was refluxed on a water-bath for 10 hours. The material was then poured into a basin and was diluted with water when a solid separated out. It was filtered, washed with water and dried. Yield 4·57 gm. The hydrolysed product was extracted with 500 c.c. of ether and the ether extract was shaken with 500 c.c. of 10 per cent caustic soda solution when a voluminous white solid separated. It was filtered, and washed with ether and water. The solid material was suspended in water and acidified with dilute hydrochloric acid. The free acid was filtered, washed with water and dried, m.p. 274–285°; yield 3·8 gm.

For purification it was charcoalized in ethanol (95%) and crystallized repeatedly from ethanol, m.p. 304–307°. For analysis the sample was dried in vacuum over P_2O_5 at 140° for 10 hours.

Found: C, 78·91; H, 10·55
Calc. for $C_{30}H_{48}O_3$: C, 78·95; H, 10·53%

The ether extract of the sapogenin, after removal of the acid fraction, was washed with dilute hydrochloric acid and water and was dried over anhydrous sodium sulphate. The solvent was removed when a viscous pale yellow mass was obtained. Yield 350 mg.

The alcoholic extract of the seeds, after removal of the solid saponin by filtration, was concentrated to a syrupy mass. It was shaken with ether (500 c.c.) and the ether-insoluble substance was hydrolysed with alcoholic hydrochloric acid as in the previous case. On working up, an acid (3 gm.) and a viscous yellowish neutral fraction (300 mg.) were obtained. The acid was purified as before and was found to be identical with the acid previously obtained.

The combined neutral sapogenin fraction was dissolved in 10 c.c. of benzene and was adsorbed in a column of Brockmann alumina (16 cm. × 1·2 cm.). It was eluted with 100 c.c. of petroleum ether (40–60°) when a small amount of yellow viscous mass could be obtained on evaporation of the solvent. It was again eluted with 300 c.c. of petroleum ether-benzene mixture (2 : 1) when a further amount of a yellowish viscous mass was obtained. Elution with further quantity (130 c.c.) of the same solvent mixture gave, on evaporation, a white solid residue (110 mg.), m.p. 160–170°. Elution was then continued with benzene but no further quantity of material could be obtained. The solid material was crystallized repeatedly from aqueous ethanol; m.p. 176-177°.

Found: C, 78·92, 78·96; H, 10·71, 10·68
$C_{30}H_{48}O_3$ requires C, 78·95; H, 10·53
$C_{30}H_{50}O_3$ requires C, 78·6; H, 10·92%

Preparation of the Monoacetyl Derivative of the Acid

The acid (500 mg.) was heated for two hours with pyridine (15 c.c.) and acetic anhydride (20 c.c.) and the reaction product was poured into crushed ice and the solid material, which separated, was filtered, washed with water and crystallized from ethanol; m.p. 259–261°.

Found: C, 76·99; H, 10·25
Calc. for $C_{32}H_{50}O_4$: C, 77·11; H, 10·04%

Preparation of the Methyl Ester of the Acid

The acid (2 gm.) was dissolved in 300 c.c. of ether and was cooled in ice. To this was added slowly an ethereal solution of diazomethane in excess and the product was worked up in the usual way. The crude product was dissolved in benzene (20 c.c.) and adsorbed in a column of acid washed alumina (48 cm. × 1·6 cm.) and was eluted with benzene. The first fraction (60 c.c.) of the elute, on evaporation, gave a very small amount of pale yellow solid, m.p. 95–123°. The succeeding fractions (total 200 c.c.) furnished a solid of almost constant melting point, 190–198°. Elution was continued with acetone but no further quantity of material could be obtained.

The fraction melting at 190–198° (1·8 gm.) was crystallized from acetone and subsequently from alcohol, m.p. 198–200°. The m.p. of mixture of the ester and methyl oleanolate was also 198–200°.

Found: C, 79·17; H, 10·56
Calc. for $C_{31}H_{50}O_3$: C, 79·15; H, 10·64%

Preparation of the Acetyl Derivative of the Methyl Ester

The methyl ester (100 mg.) was heated on a water-bath for two hours with pyridine (5 c.c.) and acetic anhydride (5 c.c.) and the product was worked up in the usual way and crystallized from aqueous ethanol; m.p. 220–222°.

Found: C, 77·39; H, 10·31
Calc. for $C_{33}H_{52}O_4$: C, 77·34; H, 10·16%

SUMMARY

From the seeds of *Luffa cylindrica* Roem. a crystalline saponin has been isolated which on hydrolysis gives oleanolic acid and a neutral sapogenin, $C_{30}H_{48-50}O_3$, m.p. 176-177°.

ACKNOWLEDGEMENT

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V. CONSTITUENTS OF THE GALLS OF *PISTACIA INTEGERRIMA*: STUDIES IN THE STRUCTURE OF TWO TRITERPENE ACIDS, PISTACIENONIC ACIDS A AND B

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Pistacia integerrima Stewart (Fam. Anacardiaceae) is a tall, nearly glabrous tree, commonly met with in the outer ranges of the north-western Himalayas. Peculiar gall-like excrescences which give the appearance of horns from a distance are formed, by insect bites, on the leaves and petioles of this plant and hence the vernacular name *Kakrasringi*. The galls, which vary in size, are of a pale greenish-brown colour, with a reddish inner surface covered with the debris of the insects and their excrementitious matter. The galls have been highly valued in Indian medicine as a useful remedy for asthma, phthisis and other ailments of the respiratory tract.

Chopra and Ghosh[1] reported the presence of a crystalline hydrocarbon of m.p. 146° in the galls. Karimullah[2] *et al.* and Ghosh[3], who further examined the galls, isolated two crystalline compounds of m.p. 180° and 161° respectively and while the former thought they were non-nitrogenous hydroxy compounds, the latter suggested from analysis and colour reactions that they were triterpene acids of the molecular formula $C_{30}H_{44}O_3$. Ghosh (*loc. cit.*) also obtained the same methyl ester of m.p. 108°-109°, by methylation of each of the acids with dry methanolic hydrogen chloride at 0°. As stated in an earlier communication[4] on the subject and progress reports[4a] of this Institute, we took up a re-investigation of the constituents of the galls in order to study the structure of these acids and to locate other compounds, if any.

The powdered galls were extracted with boiling petroleum (b.p. 60°–80°) and the residue separated into neutral and acidic fractions. The neutral fraction on chromatography over alumina gave a waxy substance of m.p. 75°–77°, β-sitosterol and a triterpene alcohol, most probably tirucallol, in low yield.

Methylation of the acid fraction followed by chromatographic separation gave crystalline methyl esters of the two triterpene acids of Ghosh (*loc. cit.*) now called pistacienonic acid A and pistacienonic acid B. The methyl esters gave identical analytical data and were found to conform to either $C_{31}H_{48}O_3$ or $C_{32}H_{50}O_3$. However, the analysis of pistacienonic acid A with six of its derivatives is in better agreement with a C_{30}-formulation for the acid, namely $C_{30}H_{46}O_3$.

Hydrolysis of the methyl esters with 5% alcoholic potassium hydroxide gave almost quantitative yields of the acids showing that the carboxyl group is unhindered in both.

Contrary to the findings of Karimullah *et al.* (*loc. cit.*) no hydroxyl groups are present in the acids, as the methyl esters remained unchanged on acetylation, nor could any hydroxy fraction be isolated from the acids by treatment with Girard[5] reagent *P*. Pistacienonic acid A, however, gave an *iso*-acid with hot acetic anhydride and pyridine.

Both the acids are keto-carboxylic acids, the methyl esters forming oximes and giving the corresponding hydroxy compounds on reduction with sodium borohydride. Lithium aluminium hydride reduction of methyl pistacienonate A gave the expected pistaciénediol A. The methyl esters gave a yellow colour with tetranitromethane and a positive Zimmermann test[6], characteristic of 3-keto group in triterpenoids. Methyl pistacienolate A on oxidation with chromium trioxide-pyridine complex according to Sarett's method[7] gave back the keto ester.

The infrared absorption spectra of methyl pistacienonates A and B, which are closely allied, showed bands at 1706 cm.$^{-1}$ (superimposed six-ring ketone and carbomethoxy group) and at 1645 cm.$^{-1}$ and 1605 cm.$^{-1}$ (double bond). Other bands present are at 1365 and 1385 cm.$^{-1}$ (*gem*-dimethyl group) and at 1438 and 1150 cm.$^{-1}$. Curves for infrared spectra of the methyl esters are shown in Figs. 1 and 2.

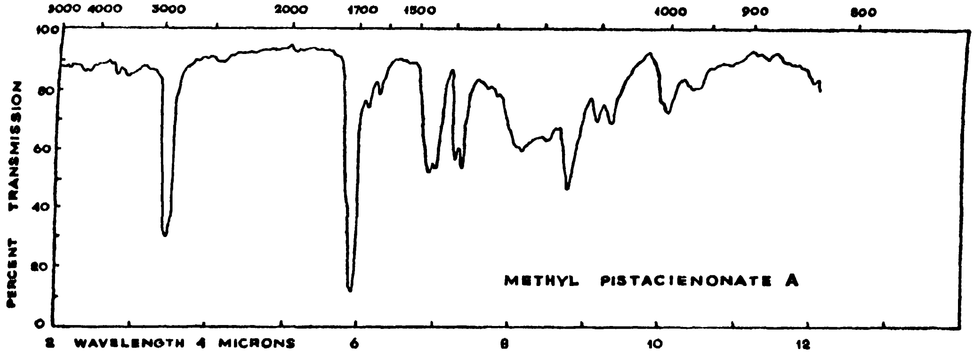


FIG. 1

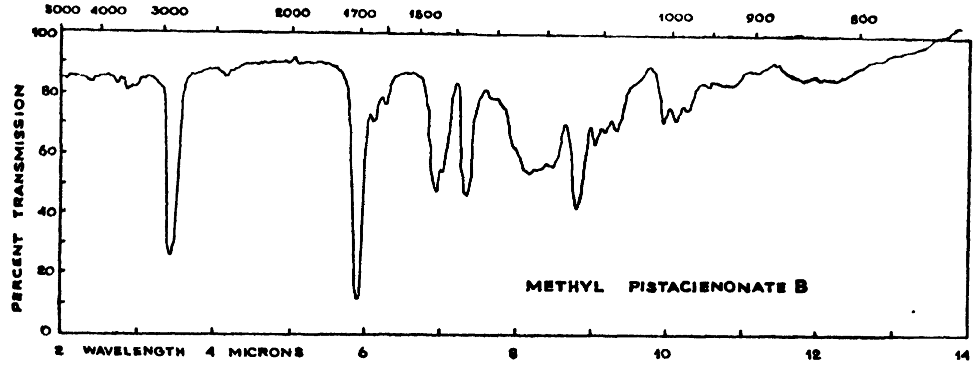


FIG. 2

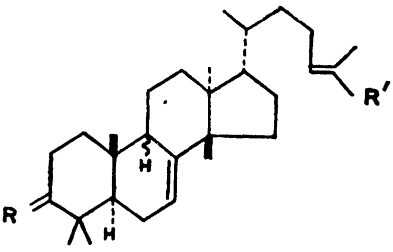
Methyl pistacienonate A and the acetyl derivative of methyl pistacienolate A consumed two moles of perbenzoic acid in 24 hours, showing the presence of two reactive double bonds in them. Methyl pistacienonate B on the other hand contained only one double bond reactive to per-acid and since its molecular formula and other properties are so close to the other methyl ester, a second double bond, sterically more hindered, may also be present in it. The methyl esters showed no selective absorption in the ultraviolet above 2200 Å, proving the absence of a conjugated diene system.

The formula $C_{31}H_{48}O_3$ for methyl pistacienonate A with two double bonds suggests a tetracyclic structure. The molecular rotation differences[8] of acetyl and keto groups show very close similarity to those of butyrospermol (I), the structure[9] of which has been established recently. The negative shift in the molecular rotation on conversion of the alcohol to the keto group is so characteristic of the butyrospermol series, that a possible relationship of pistacienonic acid A to butyrospermol is indicated.

	M_D					
	Alcohol	Acetate	Keto	ΔAcetate	ΔKeto	Ref.
Butyrospermol	−51°	+51°	−170°	+102°	−119°	*a*
Methyl pistacienonate A	−213	−121	−334	+92	−121	*b*
Pistacienonic acid A	−211	—	−354	—	−151	*c*

a—Heilbron, Jones and Robins, *J. Chem. Soc.*, 1949, 444.
b, *c*—this paper.

This suggests a tetracyclic formulation for pistacienonic acid A, closely related to butyrospermol (I), and the structure (II) may be tentatively proposed for the acid A, in view of the unhindered nature of the carboxyl group. With the idea of degrading the molecule by epoxidation of the double bond and subsequent cleavage with periodic acid, the epoxide of acetyl methyl pistacienolate A was prepared and was hydrolysed with boiling 1% ethanolic 2N-sulphuric acid.[10] But neither the epoxide nor the hydrolysed product could be crystallized.



I, $R = <^{H}_{OH}$, $R' = CH_3$
II, $R = O$, $R' = CO_2H$

As a possible double-bond isomerism might be existing between methyl pistacienonate A and methyl pistacienonate B, the former was tried to be converted into the latter under acid catalysed conditions.[11] The product obtained yielded no crystalline solid.

For the confirmation of the above structure (II), attempts were again made to convert the carboxyl function of the acid or its suitable derivative into a methyl group. The methyl ester was reduced with lithium aluminium hydride. The diol so obtained was further oxidized with chromic oxide-pyridine complex according to Sarett (*loc. cit.*). This afforded a mixture of neutral compounds, which did not yield to purification and hence the proposed further Wolf-Kishner reduction of the keto-aldehyde had to be abandoned.

At this stage we noticed two communications by Barton and Seoane[12] and Seoane[13] on the structure of masticadienonic acid and its isomer, isolated from gum mastic, which they established as tirucall-7-en-27-oic acid and tirucall-8-en-27-oic acid respectively. The published physical constants of these acids and some of their derivatives are very close to those of our pistacienonic acids A and B (see p. 31).

EXPERIMENTAL

Rotations were determined in $CHCl_3$ at room temperature. M.p.s were determined in sulphuric acid bath and are uncorrected. The alumina used for chromatography was of neutral Brockmann type. Light petroleum refers to the fraction with b.p. 40°–60°.

Extraction of the Galls of Pistacia integerrima.—The air-dried powdered galls (600 g.) were extracted with boiling petroleum (b.p. 60°–80°) in a Soxhlet apparatus for 24 hours. The extract was evaporated to dryness *in vacuo* and the dark green viscid mass (47 g.) was dissolved in ether (500 c.c.) and extracted with sodium hydroxide solution (4%). The acidic fraction was redissolved in ether (300 c.c.) and re-extracted with 1% alkali (1 lit.). The alkali layers on acidification with dilute hydrochloric acid yielded a fawn-coloured crystalline solid which was filtered off and washed with minimum amount of methanol. The resulting almost colourless acid mixture (21 g.) had an extended m.p. of 145°–165°. The methanol washings on concentration gave a dark brown residue which was treated separately.

Examination of Neutral Fraction.—The dark green neutral fraction was adsorbed from benzene (100 c.c.) on alumina (500 g.) and eluted successively with the usual range of solvents.

Fraction	Solvent	Product
I ..	Light petroleum (100 c.c.)	Large amount of yellow oil
II ..	Light petroleum (500 c.c.)	A colourless gum turning opaque on standing
III ..	Benzene (1,000 c.c.)	A colourless viscid mass
IV ..	Benzene-ether (1 : 1 ; 300 c.c.)	A yellow gum
V ..	Benzene-ether (1 : 9 ; 500 c.c.)	A yellow gum

The dark yellow oil from fraction (I) was dissolved in hot acetone and cooled. A colourless waxy solid (120 mg.) of m.p. 70°–74° was obtained which on further crystallizations from acetone melted at 75°–77°.

The acetone mother-liquors, on concentration, gave a yellow oily residue, which was insoluble in methanol. This was refluxed with ethanolic potassium hydroxide (50 c.c.; 5%) for 3 hours, alcohol distilled off and the residue diluted with water. Ether extraction gave a small amount of gum which was adsorbed from benzene (10 c.c.) over alumina (50 g.). Elution with light petroleum (150 c.c.) gave a yellow mass, insoluble in methanol, which was obviously the unhydrolysed oil. Further elution with benzene (200 c.c.) gave a colourless gum, from which, on crystallization from methanol, a powdery solid of m.p. 122°–132° was obtained. This gave Liebermann-Burchardt reaction, the colour changing from pink to blue. The amount was found to be too small for further investigation.

Triterpene Alcohol.—A colourless gum was obtained from Fraction (II). This was boiled with methanol and the insoluble oily residue filtered off. The methanol solution on concentration first deposited a small waxy solid of m.p. 70°–90°, which was rejected. A second crop of crystalline solid of m.p. 121°–127° was obtained, which on further crystallizations from aqueous ethanol afforded colourless needles, m.p. 129°–131°. It gave, in the Liebermann-Burchardt reaction, an orange colour changing to deep red with a green fluorescence.

The solid (100 mg.) was acetylated with pyridine (5 c.c.) and acetic anhydride (5 c.c.) on the steam-bath for 2 hours and worked up in the usual way. The residue was adsorbed from benzene (3 c.c.) over alumina (20 g.) and eluted with light petroleum. Crystallization from rectified spirit gave the acetyl of a triterpene alcohol, m.p. 159°–161°. (Found: C, 81·77; H, 10·93. $C_{32}H_{52}O_2$ requires C, 82·05; H, 11·1%). It gave a yellow colour with tetranitromethane.

The acetyl derivative (30 mg.) on hydrolysis with boiling ethanolic potassium hydroxide (10 c.c.; 5%) and crystallization from aqueous ethanol gave back the triterpene alcohol, m.p. 132°-133°. The molecular formula and the m.p.s of the triterpene alcohol and its acetate agree with those of tirucallol.

β-Sitosterol.—The residue (150 mg.) from the benzene Fraction (III) on repeated crystallizations from methanol gave colourless plates, m.p. 137°-138°. It gave the Liebermann-Burchardt test, the colour changing from pink to blue. Mixed m.p. with an authentic sample of β-sitosterol was undepressed. Acetylation of the substance (50 mg.) with pyridine (3 c.c.) and acetic anhydride (3 c.c.) gave an acetate of m.p. 124°-125°, which was undepressed with an authentic sample of β-sitosterol acetate.

The combined benzene-ether Fractions (IV and V) gave a yellow gum which did not crystallize. Even on acetylation and chromatography, no crystalline solid could be obtained.

Isolation of the Methyl Esters of the Acids.—The acid mixture (12·5 g.) of m.p. 145°–165° was methylated with an excess of ethereal diazomethane and the resulting mixed esters were crystallized from rectified spirit. A colourless solid of m.p. 119°–121° was obtained which on two further crystallizations from aqueous ethanol gave methyl pistacienonate A (3·7 g.) as colourless needles, m.p. 125°, $[\alpha]_D - 71·4°$ (*c*, 0·95). (Found: C, 79·74; H, 10·20. $C_{31}H_{48}O_3$ requires C, 79·49; H, 10·26%). Methyl pistacienonate A gave a yellow colour with tetranitromethane and in the Liebermann-Burchardt test a deep red colour with green fluorescence. It showed no selective absorption in the ultraviolet above 2200 Å. It gave a weak colour in the Zimmermann test.

3·5 g. of the remaining mixed esters, m.p. 95°–100°, were adsorbed from light petroleum-benzene (4 : 1 ; 75 c.c.) over alumina (210 g.). Elution with light petroleum-benzene (4 : 1 ; 1,000 c.c.) gave methyl pistacienonate B (0·9 g.) which crystallized from aqueous ethanol as needles, m.p. 108°–110°, raised by further crystallizations to 112° ; $[\alpha]_D + 26°$ (*c*, 0·89). (Found: C, 79·71 ; H, 10·36. $C_{31}H_{48}O_3$ requires C, 79·49; H, 10·26%). This gave the same colour reactions as methyl pistacienonate A, except that the colour in the Zimmermann test was very pronounced. Further elution with light petroleum-benzene (4 : 1 ; 500 c.c.) gave a gum which on crystallization from aqueous ethanol melted at 95°–105° and hence the column was completely washed down with ether, and the whole residue was re-adsorbed from light petroleum-benzene (1 : 1) on alumina (200 g.) and the eluted fractions were collected as follows:

Fraction	Solvent	Product
I	Light petroleum-benzene (3 : 2 ; 600 c.c.)	Colourless gum
II	Light petroleum-benzene (3 : 2 ; 400 c.c.)	Colourless gum
III	Light petroleum-benzene (1 : 1 ; 600 c.c.)	Solid, m.p. 115°–121°
IV	Benzene (1,000 c.c.)	Solid, m.p. 118°–123°
V	Benzene-ether (1 : 9 ; 1,000 c.c.)	Yellow gum

Fraction I on crystallization from aqueous ethanol gave 400 mg. more of methyl pistacienonate B, m.p. 108°–110°.

Fraction II crystallized from rectified spirit. 200 mg. of methyl pistacienonate A of m.p. 121°–123° was obtained from this.

Fractions III and IV were combined and on crystallization gave 300 mg. of pure methyl pistacienonate A.

Fraction V could not be crystallized.

Pistacienonic Acid A.—Methyl pistacienonate A (500 mg.) was refluxed for 3 hours with ethanolic potassium hydroxide (50 c.c.; 5%) on the steam-bath. Dilution with water and extraction with ether removed only traces of the unchanged ester. The alkaline solution on acidification gave pistacienonic acid A, which crystallized from methanol or aqueous ethanol as needles, m.p. 173°–176°, raised by further crystallizations to 175°–178°;

$[\alpha]_D -80°$ (*c*, 0·89). (Found: C, 79·49; H, 10·55. $C_{30}H_{46}O_3$ requires C, 79·3; H, 10·13%).

Pistacienonic Acid B.—Hydrolysis of methyl pistacienonate B (300 mg.) with ethanolic potassium hydroxide (30 c.c.; 5%) as described above, and crystallization of the product from methanol or aqueous ethanol, gave pistacienonic acid B as prisms or laminas, m.p. 158°–161°. (Found: C, 79·13; H, 10·14. $C_{30}H_{46}O_3$ requires C, 79·3; H, 10·13%).

Fractionation of the Acid Mixture.—The mixed acids, m.p. 145°–165°, were dissolved in methanol and the solution was slowly concentrated at room temperature. Two types of crystals, needles and prisms, were deposited. They were mechanically separated and repeatedly crystallized from methanol, when the pure acids identical with the above-mentioned pistacienonic acid A and pistacienonic acid B were obtained.

Methylation of the acids with ethereal diazomethane, followed by chromatographic purification gave methyl pistacienonate A and methyl pistacienonate B identical with the above samples.

Attempted Acetylation of the Methyl Esters.—Methyl pistacienonate A (250 mg.) in pyridine (5 c.c.) and acetic anhydride (5 c.c.) was heated on a steam-bath for 2 hours. After working up in the usual way and crystallization from methanol, the unchanged compound, m.p. 121°–123°, was got back.

Acetylation of methyl pistacienonate B, as described above, gave back only the starting material, m.p. 108°–110°.

Acetylation of Pistacienonic Acid A.—Pistacienonic acid A (200 mg.) was heated on a steam-bath for 2 hours with pyridine (5 c.c.) and acetic anhydride (5 c.c.). Crystallization from methanol gave silky needles of an *iso*-compound, m.p. 205°–207°. (Found: C, 78·64; H, 10·15. $C_{30}H_{46}O_3$ requires C, 79·3; H, 10·13%).

Treatment of the Mixture of Acids with Girard Reagent P.—The mixed acids (1·5 g.) were boiled for ¾ hour with Girard reagent P (0·5 g.) in absolute ethanol (15 c.c.) and AnalaR acetic acid (1 c.c.). The solution was poured into ice-water (100 c.c.) containing the calculated amount of alkali to neutralize 0·9 c.c. acetic acid and the mixture extracted with ether. The residue from the ether extract was insignificant showing the absence of a hydroxy fraction.

The aqueous solution on treatment with concentrated hydrochloric acid (20 c.c.) gave back the original keto-acid mixture, m.p. 145°–165°.

Oximation of the Methyl Esters.—Methyl pistacienonate A (200 mg.) in pyridine (10 c.c.) and absolute ethanol (3 c.c.) was heated under reflux for 2 hours with hydroxylamine hydrochloride (200 mg.). Dilution with water gave a solid which was filtered, washed and crystallized from aqueous ethanol. It is the oxime of methyl pistacienonate A, m.p. 137°–138°. (Found: C, 77·34; H, 10·31; N, 3·12. $C_{31}H_{49}O_3N$ requires C, 77·02; H, 10·14; N, 2·9%).

The oxime of methyl pistacienonate B was similarly prepared and had the m.p. of 201°–203°. (Found: C, 77·25; H, 10·20; N, 3·06%).

Reduction of Methyl Pistacienonate A with Sodium Borohydride.—To a solution of methyl pistacienonate A (500 mg.) in dioxan (15 c.c.), sodium borohydride (125 mg.) in aqueous dioxan (1 : 1 ; 15 c.c.) was added, and the mixture was kept for 1 hour at 30°. It was then poured into excess of 10% dilute hydrochloric acid and the product isolated by means of ether. It was adsorbed from benzene over alumina (30 g.) and eluted with the same solvent. Crystallization from aqueous ethanol gave methyl pistacienolate A as shining plates, m.p. 123°–124°, $[\alpha]_D -45·4°$ (*c*, 1·19). (Found: C, 79·34; H, 10·62. $C_{31}H_{50}O_3$ requires C, 79·15; H, 10·64%).

Acetylation of Methyl Pistacienolate A.—Methyl pistacienolate A (300 mg.) was acetylated with acetic anhydride (5 c.c.) and pyridine (5 c.c.) on a steam-bath for 2 hours. The mixture was poured into water and the product extracted with ether. The ether solution was washed successively with aqueous potassium hydroxide, aqueous hydrochloric acid and water, and dried. The residue after evaporation of ether crystallized from aqueous ethanol, yielding the acetyl of methyl pistacienolate A, m.p. 127°-128°, $[\alpha]_D$ $-23{\cdot}6°$ (*c*, 0·85). (Found: C, 77·29; H, 10·15. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

Pistacienolic Acid A.—Acetyl of methyl pistacienolate A (250 mg.) was refluxed for 3 hours with alcoholic potassium hydroxide (25 c.c.; 5%). Most of the alcohol was distilled off and the solution diluted with water. Extraction with ether removed only traces of the unhydrolysed ester. Acidification of the alkaline layer with dilute hydrochloric acid gave pistacienolic acid A, crystallizing from rectified spirit as fine needles, m.p. 200°-202°, $[\alpha]_D$ $-46{\cdot}2°$ (*c*, 1·56). (Found: C, 79·06; H, 10·45. $C_{30}H_{48}O_3$ requires C, 78·95; H, 10·53%).

Perbenzoic Acid Titration of Methyl Pistacienonate A and Acetyl of Methyl Pistacienolate A.—To methyl pistacienonate A (43·8 mg.) dissolved in pure chloroform (5 c.c.) at 0°, a solution of perbenzoic acid in chloroform (5 c.c.; 0·2 N) was added. The mixture was kept at 0°C. and 0·5 c.c. samples were withdrawn every day and treated with a mixture of potassium iodide (0·5 g.) and acetic acid (3 c.c.) in water (20 c.c.) and then titrated with 0·0188N sodium thiosulphate solution, starch being used as indicator. A blank determination was carried out at the same time and under the same conditions. After one day 1·9 moles of perbenzoic acid were consumed. After two to three days it was only 2·1 moles.

Acetyl of methyl pistacienolate A (34·3 mg.) in chloroform (5 c.c.) was treated with a solution of perbenzoic acid (5 c.c.; 0·2 N) at 0° as in the above experiment. After one day 1·97 moles of perbenzoic acid were consumed and the figure did not change on the 2nd day.

Hydrolysis of the Epoxide from Acetyl of Methyl Pistacienolate A.—Acetyl of methyl pistacienolate A (348 mg.) in chloroform (5 c.c.) was kept at 0° for 24 hours with 2 moles of perbenzoic acid in chloroform (5 c.c.; 0·2 N). Chloroform was distilled off *in vacuo*, the residue taken up in ether and the ether extract washed with sodium bicarbonate solution and water, dried and evaporated. The epoxide obtained could not be crystallized. It was boiled under reflux for 60 hours with a mixture of 2N-sulphuric acid (10 c.c.) and ethanol (90 c.c.). Afterwards most of the alcohol was distilled off, excess dilute sulphuric acid added and the product isolated with the help of ether. It was adsorbed from benzene (15 c.c.) over alumina (30 g.) and eluted with benzene-ether (1 : 1; 200 c.c.). A yellow gum highly soluble in most of the common organic solvents was obtained.

Oxidation of Methyl Pistacienolate A.—To pyridine (4 c.c.) cooled at 10° was added chromium trioxide (150 mg.) in portions with stirring. The anhydride first dissolved giving a red coloured solution and after five minutes a yellow solid began to separate out. To this slurry was added methyl pistacienolate A (150 mg.) in pyridine (4 c.c.) and kept at room temperature for 16 hours. The mixture was then treated with methanol and poured into water when a solid was thrown down. The solution with the solid was extracted with ether and the ether extract washed successively with aqueous potassium hydroxide (4%), dilute hydrochloric acid (5%) and water. Evaporation of ether and crystallization of the residue from methanol gave methyl pistacienonate A, m.p. 123°-125°, identical with an authentic sample.

Titration of Methyl Pistacienonate B with Perbenzoic Acid.—A solution of perbenzoic acid in chloroform (5 c.c.; 0·35 N) was added to methyl pistacienonate B (31·2 mg.) in chloroform (15 c.c.) at 0°. The mixture was kept at 0° and samples were withdrawn and titrated as described before. After one day 0·76 mole and after three days 1·02 mole of perbenzoic acid were consumed.

Lithium Aluminium Hydride Reduction of Methyl Pistacienonate A.—Methyl pistacienonate A (500 mg.) in dry ether (150 c.c.) containing lithium aluminium hydride (1 g.) was heated under reflux for 3 hours and then left overnight. Next day water was added to decompose the excess of the reagent and dilute sulphuric acid (50 c.c.; 10%) to decompose the metal complex. The ethereal solution was washed thoroughly with dilute sulphuric acid, sodium bicarbonate solution and water, dried and evaporated. The residue was crystallized from benzene giving pistacienediol A, m.p. 182°–184°. (Found: C, 81·21; H, 11·24. $C_{30}H_{50}O_2$ requires C, 81·44; H, 11·3%).

Acetylation of Pistacienediol A.—Pistacienediol A (60 mg.) in pyridine (3 c.c.) and acetic anhydride (3 c.c.) was acetylated on steam-bath in the usual way. A gummy residue was obtained which yielded no solid on chromatography and crystallization.

Sarett's Method of Oxidation of Pistacienediol A.—Pistacienediol A (150 mg.) was oxidized with chromium trioxide (150 mg.) in pyridine (3 c.c.) for 15 hours at room temperature. Working up, as in the case of methyl pistacienolate A, furnished a product which was adsorbed from benzene over alumina (20 g.) and eluted with benzene-ether (1 : 1). A yellow uncrystallizable gum resulted.

Hydrogenation of Methyl Pistacienonate A.—Methyl pistacienonate A (250 mg.) in acetic acid (20 c.c.) was hydrogenated at 30° in the presence of Adam's catalyst (25 mg.). After filtration and evaporation, the product was adsorbed from benzene (10 c.c.) on alumina (30 g.). Elution with benzene (150 c.c.) gave the hydrogenated product as colourless needles from methanol, m.p. 88°–90°. It gave a faint colour with tetranitromethane. Further elution with benzene-ether (3 :1; 100 c.c.) gave a gum which did not solidify.

Acid Isomerization of Methyl Pistacienonate A.—A mixture of methyl pistacienonate A (0·5 g.) in 'AnalaR' benzene (5 c.c.), concentrated sulphuric acid—'AnalaR' acetic acid (15 : 85; 50 c.c.) was kept at room temperature for two days. After dilution with water, it was extracted with ether and after washing with sodium hydroxide solution, the ether was removed. The residue was adsorbed from benzene over alumina and eluted with benzene-light petroleum (1 :2). Only a colourless viscid uncrystallizable mass was obtained.

Reduction of Methyl Pistacienonate B with Sodium Borohydride.—Methyl pistacienonate B (400 mg.) in methanol (25 c.c.) was treated with sodium borohydride (125 mg.) and kept for 1 hour at room temperature. After addition of excess of dilute hydrochloric acid (10%), the product was taken up in ether and the residue from ether adsorbed from benzene (10 c.c.) over alumina (30 g.). Elution with benzene-ether (3 : 1 ; 150 c.c.) gave methyl pistacienolate B, m.p. 115°–118°, raised by further crystallization from aqueous ethanol to 118°-119°.

SUMMARY

The galls of *Pistacia integerrima* Stewart have been found to contain a waxy compound of m.p. 75°–77°, β-sitosterol and a triterpene alcohol, most probably tirucallol in addition to the two triterpene acids already reported. The two acids, pistacienonic acid A and pistacienonic acid B, are keto-carboxylic acids of the same formula $C_{30}H_{46}O_3$ and are closely allied. Studies in the constitution of these acids have been made.

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VI. INVESTIGATIONS ON THE PHYSIOLOGICAL FACTORS IN INDUCED MUTAGENESIS

I. EFFECT OF WATER ON THE MUTAGENIC CAPACITY OF X-RAYS

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INTRODUCTION

In 1927, Müller discovered X-rays as a fruitful source of inducing rearrangement of parts of chromosomes by his exhaustive experiments on *Drosophila*. Since then, extensive work has been done on this line in both plants and animals. Goodspeed (1929), Goodspeed and Avery (1930) on *Nicotiana*, Horlacher and Killough (1933) on *cotton*, Katayama (1935) on *wheat*, Navashin (1931) on *Crepis*, De Mol (1930) on *Hyacinthus* and *Tulips*, Levitsky and Araratian (1932) on *Secale*, *Vicia* and *Crepis* and Catcheside (1935) on *Oenothera* are some of the early workers in this line, in plants.

The genetical and cytological changes induced by irradiation were mainly gene mutations, translocations, duplications and deficiencies following chromosome breakage. McClintock (1931) observed deficiencies, translocations and inversions in irradiated *Zea mays* from a study of the pachytene stages. Stone (1933) and Mather (1934) found a great number of fragmentation in the metaphase and anaphase cells which, according to them, lead to inversions and translocations by the rejoining of the broken parts.

As regards the mode of action of X-rays, it is suggested that the changes induced are due to the direct bombardment of the highly energetic secondary electrons due to primary X-rays (Catcheside, 1936). This may alter the constitution of the genes or break the chromosomes at any locus. For workers in this line, it is a common observation that all the cells exposed to uniform bombardment do not respond exactly in the same manner or degree. This necessitates further investigations on the effects of X-rays on the cells under different physiological conditions, so as to determine whether the direct bombardment of the electron can be minimized or increased according to the nature of the cell at the time of irradiation. Very few investigations have been done to find out the effect of X-rays in different environments. The results so far obtained by different workers are contradictory and hence it was considered necessary to conduct further investigations.

The present set of investigations were started to find out the effect of various physiological factors on the action of X-rays on the plant cells. In the first instance, the effect of moisture as the factor has been worked out.

MATERIALS AND METHOD

The experiments have been carried out on *Oryza sativa* L. var. *Rupsail*, obtained from the multiplication of the stock procured earlier from the Rice Research Institute, Cuttack. These seeds were treated with X-rays at different levels of moisture, viz. :—

(1) Desiccated.
(2) At atmospheric level of moisture.
(3) Soaked.
(4) Germinated.

Variation in the moisture content ranged from 7% to 26%.

METHODS

The following were the treatments given: (1) Seeds were kept for desiccation for 90 hours in a desiccator with calcium chloride. Some were used for exposing to X-rays, while the rest were used for determining the moisture content. (2) Seeds were kept for desiccation for 45 hours and treated with X-rays. (3) Seeds, kept at atmospheric humidity and temperature, were treated with X-rays. (4) Seeds were soaked in water for 12 hours and then exposed to X-rays. (5) Seeds were soaked for 24 hours in water and then irradiated. (6) Germinating seeds were also treated.

These seeds were exposed to X-rays with the help of a 'Philips Contact and Cavity Therapy Tube' working at 50 kV and 2 mA, without any filter and at a distance of 12 cm. from the target. The following doses were given in each case: 1,000 r, 2,500 r, 5,000 r and 10,000 r.

In all the cases the moisture content of the seeds at the time of irradiation was determined. For this, the seeds were kept for drying in an electric oven at a temperature of 90°C. for overnight, and at 100°C. for another 24 hours, and the difference between initial and final weights was determined. From this the percentage of moisture content in the particular set of seeds was calculated.

After irradiation the seeds were allowed to germinate on moist filter papers in petri dishes. The percentage of germination and the time required was noted. Some of the seeds were allowed to grow in pots to compare the growth rate in all cases as well as the meiotic disturbances, if any.

For cytological investigations root tips were fixed in conc. HCl and rectified spirit (1:3) for 30 minutes, and rinsed in 45% acetic acid. The root tips thus fixed were squashed and stained in iron-aceto-carmine, which gave good results both by way of staining as well as in the morphology of chromosomes. For sections, root tips were fixed in chromic-formalin (2:3) and stained with crystal violet. Attention was particularly paid to the metaphase and anaphase stages. Most of the scoring was done from the squash preparations.

OBSERVATIONS

The seeds germinated on the third day in all cases except for the desiccated seeds, which took four days. The percentage of germination was also very low in the desiccated seeds. The percentage of germination in all cases is shown in Table I.

TABLE I

Percentage of germination

Treatments	Desiccated for 90 hrs. *(7·96%)	Desiccated for 45 hrs. *(10·37%)	Ordinary *(11·38%)	Seeds soaked for 12 hrs. *(19·96%)	Seeds soaked for 24 hrs. *(26·28%)
Control ..	16	16	98	85	60
1,000 r ..	0	0	90	34	52
2,500 r ..	8	8	94	41	42
5,000 r ..	8	8	94	40	60
10,000 r ..	12	12	86	38	44

* Moisture content of the seeds at the time of treatment.

In the desiccated seeds the percentage of germination was very low, and so the seedlings could not be obtained for growing in pots for meiotic study.

In the case of seeds soaked in water for 24 hours and then treated with 5,000 r and 2,500 r, there were two cases each in which the normal growth of root was suppressed (Fig. 14). In one set of seeds, from the seeds treated with 10,000 r, a single case of polyembryony was noted (Fig. 15). In the former case the seedlings died after three days while in the latter it survived for one week.

Weekly records of the growth of all the plants were maintained. There was a marked difference in the height of plants treated with the different dosages, more so in the case of dry seeds.

TABLE II

Weeks	Control	1,000 r Ht. in cm.	2,500 r Ht. in cm.	5,000 r Ht. in cm.
1	4·72	5·08	5·18	4·55
2	17·57	19·42	17 28	15·3
3	30·7	32·57	21·63	25·8
4	43·46	44·23	32·9	40·04
5	54·84	56·65	38·73	46·2
6	67·6	71·1	51·84	61·29
7	74·7	78·4	57·75	69·61
8	77·66	86·98	64·0	81·6
9	90·52	97·31	76·5	90·79
10	98·40	104·37	87·48	95·25
11	103·09	105·58	97·43	99·49
12	105·82	112·19	98·80	105·25
13	108·71	112·3	102·87	108·61
14	109·24	114·173	104·52	111·25
15	112·13	116·89	105·84	112·01
16	117·27	121·28	112·85	118·41
17	117·602	126·746	113·99	119·60

Table II shows the average height of 20 plants in each case at the end of each week. During the first two weeks there was no marked variation in the vegetative activity of the plants. But after two weeks the growth was accelerated in 1,000 r and retarded in the case of 2,500 r, which lasted up to the seventh week. Then there was the recovery in the case of 2,500 r, so that at the time of flowering the plants attained almost similar heights in all the cases.

Graph 1 shows the effect of X-rays on the vegetative activity of the plant. Fig. 16 shows the marked difference in the growth of the plants in the different treatments after a few weeks. There were a few chlorophyll mutants in all the treated lots. They included 'xantha or lethal yellow' where the leaves were pale yellow. They lived only for a week after transplanting. There were a few plants which had green leaves first, which later turned yellow. These plants also died after five weeks. Such chlorophyll mutants have been called 'Lutescent' (Ramiah and Parthasarathy, 1938).

CYTOLOGICAL BEHAVIOUR

The cytological behaviour of the control as well as the treated plants was studied from squash preparations.

I. *Seeds desiccated for 90 hours.*—Here the water content was 7·96 %.

1,000 r: Twenty-five seeds were kept for germination after irradiation. The seeds were very hard and did not germinate and hence no investigations could be undertaken.

2,500 r: Dividing cells were seen only occasionally. Only 11 anaphase cells could be seen out of three slides prepared and a single case of bridge was observed.

An interesting observation made during the present study was a mitotic cell resembling a meiotic stage. Fig. 2 shows the chromosomes split to form loop-like structures resembling the chiasmata at the diakinesis. Fourteen chromosomes were looped, while in eight others it took the form of 'V' and only two chromosomes were normal. The nature and the causes of the occurrence of this phenomenon will be discussed later.

5,000 r: The cell division was almost normal. Out of the 76 anaphase cells examined, only two cells showed aberrations; one a bridge and the other an acentric fragment.

10,000 r: Only 32 anaphase cells could be seen. There were two aberrant cells. Out of 55 metaphase cells scored, only one showed a fragment.

In general, the cells showed a tendency to clump together in all the irradiated lots. The effects of dehydration were evident from the granulate structure of the cytoplasm.

II. *Seeds desiccated for 45 hours.*—The water content after desiccation for 45 hours was found to be 10·37%. The seeds were then irradiated. Germination was very poor in all the cases.

1,000 r: Twenty-five seeds were put for germination, but none germinated and hence no investigation was possible.

2,500 r: Out of the 25 seeds treated and put for germination, only two seeds germinated. The roots were very hard at the tip and the meristematic activity was so low that only few cells were found to be dividing. No aberrations could be seen.

5,000 r: Only two seeds germinated out of the 25 seeds treated. Here also the root tips were very hard, that even after hydrolysing for one hour it was difficult to get a good squash preparation. Only 23 anaphase cells could be scored, out of which one cell with a chromatid bridge was observed.

10,000 r: Only three seeds germinated among the 25 seeds treated. Seventy-six anaphase cells were examined out of which one cell showed a fragment while another had a fragment and a bridge and three other cells with only bridges. In one cell there were two chromosome bridges. There was a metaphase plate where the chromosome number was 26 which probably may have arisen due to misdivision in the previous generation.

III. *Seeds at atmospheric level of moisture.*—The water content of the seed at the time of irradiation was found to be 11·88%.

1,000 r: Germination percentage was high. The root tip squashes showed aberrations like bridges and fragments. Eighty-three anaphase cells were examined. There were 10 cases of bridge formations arising either out of the failure of separation of the chromosomes or due to stickiness (Fig. 5). There was a metaphase cell with the somatic number 22 (Fig. 3).

2,500 r: A large number of dividing cells were scored. Out of the 362 anaphase cells studied, 24 cells showed aberrations. They included stickiness, bridges and late separation. Fragments were more frequently seen at anaphase, though in a single case a fragment was recorded at metaphase stage also.

5,000 r: The aberrations were more than that found in the previous case. Out of the 487 anaphase cells, there were 40 cells showing aberrations, mostly bridges. There were two metaphase cells having a fragment each. One anaphase cell showed a bridge and an acentric fragment (Fig. 4).

10,000 r: The percentage of aberrations were more in this case. Out of the 132 anaphase cells examined, 16 cells had bridges or fragments. Only two metaphase cells with one fragment each could be seen. The number of bridges varied in the different cells. In one cell there were four bridges, of which three were chromosome bridges and the fourth appeared to be formed due to stickiness of chromosomes. There were three bridges in another cell and one each in three other cells.

IV. *Seeds irradiated after soaking in water for 12 hours.*—The water content in this case was found to be 19·96%. The vegetative growth was not good since the treatment for the experiment was done late in the season.

1,000 r: Germination was normal. Chromosomal aberrations were seen in only a few cells. Of the 151 anaphase cells examined, six cells showed aberrations; only bridges could be seen in these aberrant cells. Two metaphase cells revealed the presence of a fragment each (Fig. 6). Clumping of the chromosomes was less prominent.

2,500 r: One hundred and twenty-nine anaphase cells were examined. Five of them were found to be aberrant cells. In one case a bridge and a fragment were both seen in the same cell, the latter being outside the spindle. There were two metaphase cells with a fragment each.

5,000 r: Only a few dividing cells could be obtained for the study. Thirty-five anaphase cells were examined, out of which two had bridges. In one cell there were two bridges (Fig. 7).

10,000 r: Only 50 dividing cells could be observed, out of which four cells were abnormal. In one case there were two bridges, one in the second and the two other cells each with a fragment. A single case of late separation was also recorded.

In general, the percentage of aberrations was less as compared with those of the dry seeds (Table III).

V. *Seeds soaked in water for 24 hours.*—The water content of the seeds at the time of irradiation was found to be 26·28%. Since the experiment was done late in the season, the germination percentage was lower. The vegetative growth also was not good, both in control and treatments.

1,000 r: The germination in this treatment was particularly low. The number of dividing cells was also few. Out of the 132 anaphase cells scored, only four cells were aberrant. In three cells there were bridges and in one a fragment. In a telophase cell one fragment was seen away from the two daughter nuclei (Fig. 9). This will lead to a loss of chromatin with the probable change in the genetic constitution.

2,500 r: One hundred and ninety-four anaphase cells were examined, of which only seven cells were aberrant. In three cells there were bridge formations, in one a bridge and a fragment and three cells with fragments only were seen. In one cell there were two fragments (Fig. 8). In one metaphase plate the number of chromosomes was 23 which may be due to misdivision in the previous generation (Fig. 17).

5,000 r: Out of the 167 cells at anaphase examined, five were abnormal. In four cells there were only bridge formations (Fig. 11), while in one there was a fragment and a bridge.

10,000 r: Two hundred and eight anaphase cells were scored in which four were abnormal. Three of these showed bridge formations, while in the fourth a small fragment was present. In two cells at metaphase there were fragments. In the polar view of one of the metaphase plates two fragments lying very close to two chromosomes were noticed (Fig. 12). One anaphase cell showed three bridges (Figs. 13 and 19).

TABLE III

Nature of seed and water content in percentage	DOSAGE											
	1,000 r			2,500 r			5,000 r			10,000 r		
	No. of cells examined	No. of aberrant cells	% of aberration	No. of cells examined	No. of aberrant cells	% of aberration	No. of cells examined	No. of aberrant cells	% of aberration	No. of cells examined	No. of aberrant cells	% of aberration
Desiccated for 90 hrs. (7·962%) ..	..	..	..	11	1	9·1	76	2	2·63	32	2	6·2
Desiccated for 45 hrs. (10·37%) ..	..	..	..	..	..	..	23	1	4·3	76	5	6·58
Ordinary seed (11·88%)	83	11	13·25	362	24	6·63	487	40	8·213	132	16	12·12
Soaked for 12 hrs. (19·96%) ..	151	6	3·907	129	5	3·87	35	2	5·71	50	4	8·0
Soaked for 24 hrs. (26·28%) ..	132	4	3·03	194	7	3·60	167	5	2·99	208	4	1·92

TABLE IV

Aberrations noted daily in irradiated seedlings

Treatments	1st day		2nd day		3rd day		4th day		7th day		9th day	
	Total No. of cells	No. of aberrant cells	Total No. of cells	No. of aberrant cells	Total No. of cells	No. of aberrant cells	Total No. of cells	No. of aberrant cells	Total No. of cells	No. of aberrant cells	Total No. of cells	No. of aberrant cells
1,000 r ..	5	1	30	1	19	0	28	1	..	..	..	..
2,500 r ..	19	1	78	2	20	4	8	2	..	..	40	2
5,000 r ..	..	..	46	3	..	..	63	2	..	..	..	..
10,000 r ..	..	..	109	8	..	..	..	..	25	4	..	..

The summary of all anaphasic aberrations in relation to water content is tabulated (Table III).

VI. Seedlings were also subjected to irradiation with the same dosages as in the case of seeds. But here the number of dividing cells were reduced.

Table IV shows the aberrations in the different sets of treatments. However, the number of dividing cells was too low for statistical analysis.

The aberrations were chiefly bridges and fragments, besides heavy clumping of the chromosomes. This clumping of the chromosomes was a frequent occurrence during the first day after irradiation.

In general, the cells were all damaged perhaps due to irradiation, and the seedlings did not survive long.

Graph II shows the influence of different water contents in the seeds on chromosome aberrations due to the action of X-rays. From the graph it will be seen that the aberrations are more in the case of dry seeds where the water content is less and are lesser in the case of soaked seeds. This difference in the percentage is remarkable when the dosage is high. In 10,000 r there is a smooth fall in the curve of the aberrations as the water content is increased.

In the case of treated desiccated seeds and the seedlings, the number of dividing cells were very few, and so the percentage of aberration was not plotted in the graph.

GENERAL CONSIDERATIONS

Generally the effects of X-rays have been classified into two: (*a*) 'primary effect' resulting in clumping and partial fusion of chromosomes and a temporary inhibition of mitotic divisions. This has been variously termed as physiological, physical and chemical effect and (*b*) 'secondary effect' characterized by chromosomal aberrations.

However, working on the cells of the corneal epithelium of *Salamander* larvae fixed at varying intervals after irradiation, Alberti and Politzer (1923, 1924) have divided them into three phases, viz.: (1) 'primary effect' characterized by a decline in the frequency of mitosis and the appearance of pycnotic nuclei with adherent chromosomes, (2) a subsequent period of inactivation and (3) a period of 'secondary effect' characterized by chromosomal aberrations.

During the primary effect, the matrix of the chromosome is altered and the mitotic mechanism is inhibited. Because of this inhibition, there is a period when most of the cells are at the resting stage. If the treatment is not too intense, the mitotic activity is revived and thereby the condensed chromosomes produce various types of induced abnormalities. But when the treatment is high, the chromonematic threads of early mitotic stages show nodal thickening along their length and the condensed chromosomes stick together or clump to form groups of chromatin matter (Marquardt, 1938). This happens perhaps due to the changes in the viscosity of the component proteins and nucleic acid. This view has also been shared by Kaufmann (1951). The change in viscosity has been explained to be due to the depolymerization of desoxyribonucleic acid.

In the present investigation, this nature could be well observed in the case of treated seedlings where the first mitosis after the irradiation showed heavy clumping. In the higher doses, the mitotic activity almost stopped. All the cells were at the resting stage at the usual time of division. The same is true in the case of treated seeds. But as 2-3 generations pass off between the time of irradiation and the germination of the seeds and the elongation of the roots to the required length, the material gets ample opportunity to revive. This revival reduces the degree of chromosome clumping which is a phenomenon of the primary effects.

The secondary effects are induced by moderate doses of radiation, where the damage is localized to chromosome breakage without weakening the synthetic process essential for mitotic and other cellular activities.

Due to the primary effect, at metaphase, the chromosomes adhere to each other and so, at anaphase, the sister chromatids separate with difficulty, resulting in bridges and fragments. These bridges may separate forming broken bridges and fragments which result in deficiency in the daughter nuclei.

Two general types of aberrations are induced by X-rays, which are chromatid breaks and chromosome breaks. The chromatid breaks are induced in the prophase stage when the chromosomes split into two sister chromatids. These are recognized by the presence of a single fragment or a single bridge accompanied by a fragment. This bridge and the fragment arise from a single break. The breaks can produce a reciprocal chromatid exchange resulting in a pseudo-chiasma formation. Sometimes the broken ends fuse to form a chromatid bridge between two chromosomes (Fig. 5) which may be sometimes accompanied by an acentric fragment.

The chromosome breakages are those induced during the resting stage when the chromosomes are in the form of single threads (Sax, 1940). This type of break is usually seen in the case of treatment with dormant seeds. These breaks include deletions, both intercalary and terminal, dicentrics, rings, translocations and inversions. The loss of a terminal portion of the arm results in a shortened arm. The acentric fragments (Figs. 4, 8, 9, 10) lie free in the cytoplasm and may be away from the spindle. In the next generation it may fuse with any one of the chromosomes or it may disorganize and be lost. The fusion between two broken ends of the chromosome results in a bridge. There were cases of two separate and independent bridges in the same cell (Fig. 7). It may be due to breaks in two different chromosomes and the fusion of the broken ends in them.

The formation of bridges suggest to a 'physiological effect' also, where due to a localized effect of the ionizing particle a general change in surface properties of the chromosome is brought about. This results in either the attachment of chromosomes where they happen to touch, resulting in 'bridges' at anaphase or to the adherence of sister chromatids which fail to separate due to some kind of stickiness (Fig. 11).

MEIOTIC PICTURE OF A MITOTIC CELL

An interesting case of a metaphase plate was noted in the case where desiccated seeds were exposed to 2,500 r. Fig. 2, as described elsewhere, is a picture of a polar view of a somatic metaphase plate, the chromosomes resembling bivalents at metaphase I of meiosis. A closer examination of the plate, however, revealed the failure of the separation of the chromatids at late metaphase either at one end or at both the ends, but the presence of the anaphasic repulsion between the chromatids caused the distancing apart of the free region, which in most of the cases was median. This gives rise to the ring-shaped structure of the chromosomes.

This failure of separation of the chromatids has been recorded not only as an effect of the irradiation but also due to other physical and chemical agencies and sometimes even under natural conditions. This phenomenon may lead to either a bridge formation or sometimes the unequal distribution at the anaphase. However, no such case of most of the chromosomes of any plate failing to separate has been recorded so far. But the more interesting feature is the failure of such separation at the ends only. There can be three possible explanations of this phenomenon.

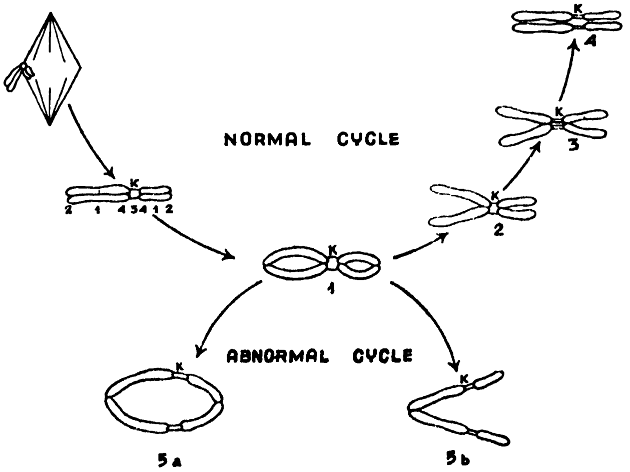
In the first place it may be caused by the stickiness of the chromosome ends. Stickiness, however, is a pathological condition, but the chances of the same due to irradiation cannot be ruled out. The stickiness of the ends may impair the separation of the tips leading to the condition observed in this case.

As an alternative, the phenomenon may be explained to be due to segmental interchange at the ends leading to the formation of the contact between the tips. This phenomenon may be compared to what has been termed as 'pseudo-chiasmata' in the somatic mitosis of *Culex*. D'Amato (1949) suggested that mitotic pseudo-chiasmata originate by a process involving the breakage and reunion of chromonemata. Darlington (1949) and Darlington and LaCour (1953) interpreted both mitotic and meiotic 'partial chiasmata' as due to errors of chromonemata, reproduction, breakage and reunion. Östergren (1948) and Levan (1949) explained that the pseudo-chiasmata occur in localized points. But, how far these views are applicable to the present observation could not be ascertained, firstly due to want of comparative morphology of the two chromatids after separation and, secondly, due to the rare occurrence of this phenomenon, as it was noted only in two cases in the present observation.

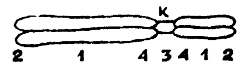
The third interpretation may be sought in the upsetting of the normal cycle of the division of the chromatids and the kinetochore. Lima-De-Faria (1956) working on the colchicine treated root tips of *Allium cepa* has shown that there exist four definite regions within a chromosome with distinct division cycle. They are in order of the occurrence—

(1) median,
(2) chromosome ends,
(3) kinetochore,
(4) most proximal region of the arms.

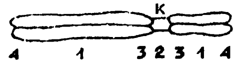
The same may be diagrammatically represented as follows:—



In other words, after the division cycles of the first and second regions are completed the double nature of the chromosome becomes clear, being joined only at the kinetochore. At this stage the chromosome is in the form of 'X-chromosome' (Lima-De-Faria, 1956). However, if due to the exposure to the X-radiations the physiology of the cytoplasm or the chromatin is so altered as to hinder the usual sequence of the division cycle, the division of the kinetochore may be brought about earlier than that of the distal end of chromosome and the chromatids may remain held together at the ends even after the separation of the proximal end (fourth stage of a normal chromosome) has been completed. This may be represented as follows:—



NORMAL



INDUCED

From this altered condition, it is easy to arrive at stages 5A and 5B of the diagrammatic cycle, representing the looped or V-shaped nature of the chromosomes, where the chromatids are held together at the two or one of the distal ends.

The first and second explanations do not seem tenable as they either involve an assumption of a pathological condition earlier, or tend to explain the phenomenon wholly on a physical basis. Biological systems are, however, much more complex, to be so simply explained. The alteration of the division cycle of the different chromosome regions, involving a physiological interpretation of a cytological phenomenon, seems to be the more probable explanation, which is in tune with the advancing knowledge of the biochemical approaches to the biological phenomena.

Behaviour of the X-ray induced chromosome breakages.—Many of the phenomena of chromosome breakage that result from any treatment, either induced by radiations or chemical, occur in the same manner irrespective of the nature of treatment.

The behaviour of the broken ends of chromosomes in the successive division is not clearly understood. Sax (1940), after analysing a number of induced chromosomal aberrations, opined that the broken ends of chromosomes either unite in the original position or produce chromosome aberrations within a short time, after the breakage due to X-rays, except for a very small proportion of breaks which appear to have lost their capacity for reunion. Stadler (1932) assumed that an acentric fragment may be included in a daughter nucleus by chance and fuse with the original broken chromosomes after several generations.

McClintock (1939) studied the behaviour of the aberrant cells in the meiosis of maize in the successive generations and found that the 'bridge-breakage-fusion-bridge' cycle is carried from one cell generation to the next in the endosperm, but in the cells of the embryo and plant tissue, the broken ends heal. Clark and Copeland (1940) also support these findings. But if the possibility of the healing of broken ends is accepted, then either the cells with deleted chromosomes are unable to compete with normal cells and fail to take part in later development or if these cells survive to remain active, chromosomes with shortened arms should be seen in subsequent generations.

From the present experiments conducted on *O. sativa*, a marked decrease in aberrations was seen as the root elongated, suggesting the probability of the aberrant cells being degenerated and getting eliminated. Only a study of the meiotic behaviour will show how far the aberrations induced by X-rays noticed in the root tips are being retained. Investigations in this line are in progress.

DISCUSSION

Effect of water on the mutagenic action of X-rays.—The biological effects of radiation have been studied since the discovery of the Röentgen rays. The interpretation of the mechanism, by which the ionization is effected, is however still speculative. The oldest of the hypotheses on this subject was the 'Poison theory' (Timofeeff-Ressorsky and Zimmer, 1947) which explains the effect of X-rays as due to the production of compounds which are poisonous to the organism. But this theory holds true only in the cases of killing and growth retarding effect.

But the study of the normal survival of the radiation affected organisms or tissues led to the enunciation of the 'Target-theory' which stated that the radiation energy is absorbed as discrete and statistically distributed processes and that the cells contain certain radio-sensitive volumes called 'target-volumes' within which the absorption processes must occur to produce biological reaction. According to this theory the mutation frequency is found to be directly proportional to radiation dose, and independent of wave-length, temperature, and radiation-sensitivity.

As the study on the mechanism of ionization progressed, it was supposed that the biological effect of radiation could be divided into 'genetical' and 'non-genetical' effects. The genetical effect is the action on genes and chromosomes while the non-genetical effect constitutes the physiological effect. The latter cannot tally with the target-theory, since physiological effect is found to depend on oxygen pressure, temperature, time-factor, water content, etc. Thus it is the result of an indirect action of the radiation. This explains that the ultimate biological effects of radiation are not the consequence of a direct absorption of energy in vital compounds, but that the changes are produced via intermediate labile compounds originating in the medium.

This explanation is supported by the findings of Thoday and Read (1947) and Giles and Riley (1950) showing the influence of oxygen on the induction of chromosome disturbances. The mutagenic effect and the existence of an indirect biological radiation effect was proved by other workers also (Stone, Wyss and Hass, 1947 ; Wagner, Haddox, Fuerst and Stone, 1950, etc.).

It has long been the common belief that germinating seeds are more sensitive to radiation than dormant ones (Stadler, 1930; Froier and Gustafsson, 1941). But Wertz (1940) and Ehrenberg (1955), after experimenting on the effects of radiation and the different physiological factors associated with the irradiation, found that radiation induced injury decreases with increasing water content of the seeds within a certain range, with a sensitivity increase at lower as well as higher water contents. Ehrenberg found the effect of varying water contents in different radiation induced properties like growth inhibition (Ehrenberg, 1955*a*) and mutation rate. He concluded that the dependence on the water content should be referred to physical and chemical rather than the physiological process.

From the present work, the same inferences can be deduced. When the seeds were desiccated and irradiated, the germination was poor and the aberrations were more. But when the seeds were soaked and irradiated the germination percentage was higher and the aberrations were less. The study of the chromosomal disturbances suggest that dry seeds are more sensitive to induction of breakages in chromosome than the soaked seeds. This sensitivity was found within a range of 7%–26% moisture. This is more pronounced in higher doses than in the lower ones.

It was also found that the inhibition of growth in the irradiated lots was confined only to a few weeks, after which there was gradual recovery. This suggests that the dependence on the water content is a physical, chemical as well as physiological process.

In short, from the present findings it is concluded that the effect of X-ray in inducing chromosomal aberrations, growth inhibition, survival and mutation was found to decrease with increasing water content within a certain range and vice versa.

SUMMARY

The present investigations have been conducted to find out the effect of various water contents on the mutagenic effect of X-rays on *Oryza sativa* var. *Rupsail.* The moisture content of the seeds has been altered by desiccation and soaking and the percentage of moisture content determined in every case. The exposure to X-radiations in each set of experiments was of the order of 1,000 r, 2,500 r, 5,000 r and 10,000 r.

The effects of X-rays on the growth of plants have been determined in terms of the height of the plants and the results presented in the form of a table. It has been found that the effect of various doses of X-rays is not directly proportional and there is an optimum dose at which the effect is maximum. Any further increase in the dose has been found to reverse the process and the deleterious effect of X-rays is reduced. The growth inhibiting effect of X-rays is considered to be a physiological one, as later in the life of the plants, this effect is reduced and the plants revive. This fact has been illustrated by means of a graph.

The effects of the various doses of X-rays on the cytological aberrations in this material have been investigated. These effects have been found to decrease proportionately with the increasing moisture content. The nature of the important cytological aberrations has been explained and their significance discussed.

The nature of the effects of X-rays both on the growth and cytological aberrations has been dealt at length in the light of the present-day knowledge. The nature of the effects on the cytology has been determined. The persistence as well as transmission of these aberrations to subsequent generations have been discussed. Further work in this line is in progress.

The relation of the degree of aberrations to the dosage of treatment has been investigated in light of the 'Target-theory' and it has been found that the theory does not hold good in the present observations on the growth of plant. In other words, no direct relationship between the effects and the target volume could be established in relation to the growth. The same holds good about the cytological abnormalities also.

Lastly, the effects of various degrees of moisture content in relation to the effects of each dose of radiation has been analysed and it has been found that the effect is proportionately lowered with the increase in moisture content.

ACKNOWLEDGEMENTS

My grateful thanks are due to Dr. K. T. Jacob, Head of the Department of Botany, Bose Institute, Calcutta, under whose guidance this work has been conducted and to Dr. D. M. Bose, Director, for the laboratory facilities and interest. My sincere thanks are also due to Prof. A. Lima-De-Faria of the Institute of Genetics, University of Lund., Stockholm, who went through some of the preparations and suggested valuable interpretations.

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EXPLANATION OF FIGURES

FIGS. 1-13 were made by using camera lucida on a Zeiss G.F. 321 binocular microscope using 100 : 13 oil immersion objective and 20× compensating eye-piece which gave 4,200× table magnification. These figures have been reduced to 2/3 size.

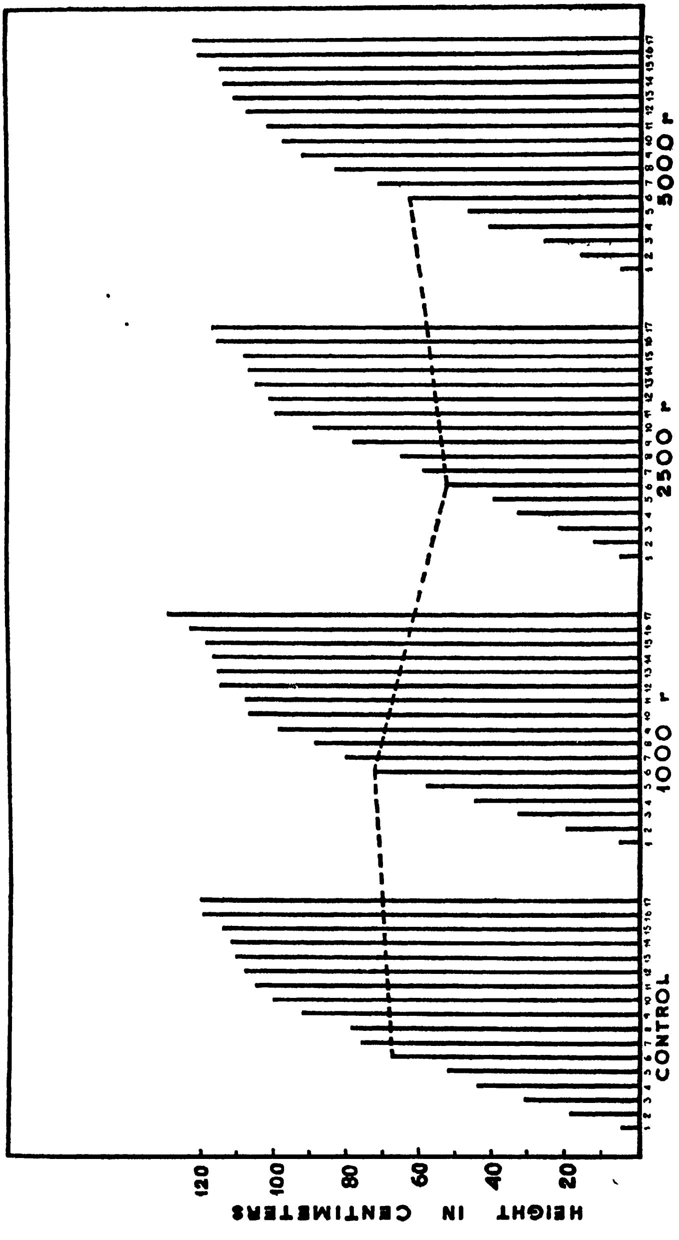
The following are the brief explanations of the figures:—

FIG. 1.	*Oryza sativa* var. *Rupsail*	—Control —Polar view of a somatic metaphase.
,, 2.	,,	2,500 r/desiccated. Polar view of a somatic metaphase showing ring-like structure following X-ray treatment.
,, 3.	,,	1,000 r/Ordinary seeds—Polar view of a somatic metaphase showing 22 chromosomes.
,, 4.	,,	5,000 r/Ordinary seeds—Somatic anaphase showing bridge and a fragment.
,, 5.	,,	1,000 r/Ordinary seed—Somatic anaphase with a single bridge.
,, 6.	,,	1,000 r/Soaked in water for 12 hours—Somatic metaphase showing a fragment.
,, 7.	,,	5,000 r/Soaked in water for 12 hours—Somatic anaphase with two bridges.
,, 8.	,,	2,500 r/Soaked in water for 24 hours—Somatic anaphase showing one fragment at each pole.
,, 9.	,,	1,000 r/Soaked in water for 24 hours—Telophase showing a fragment lagging in the middle.
,, 10.	,,	2,500 r/Soaked in water for 24 hours—Anaphase showing an acentric fragment.
,, 11.	,,	5,000 r/Soaked in water for 24 hours—Anaphase showing a bridge.
,, 12.	,,	10,000 r/Soaked in water for 24 hours—Polar view of a somatic metaphase showing two fragments.
,, 13.	,,	10,000 r/Soaked in water for 24 hours—Anaphase showing three bridges.

PHOTOGRAPHS AND PHOTO-MICROGRAPHS

FIG. 14.	Shows the root system in X-irradiated (A) and the normal seedling (B).
,, 15.	Shows polyembryonic seedling (A) and Control (B).
,, 16.	Shows the height of paddy plants in Control, 1,000 r, 2,500 r and 5,000 r.
,, 17.	Shows polar view of a somatic metaphase with 23 chromosomes.
,, 18.	Anaphase showing one bridge in the treated seeds.
,, 19.	Anaphase showing three bridges in a somatic cell of a treated seed.

GRAPH 1


HEIGHT IN CENTIMETERS
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100
80
60
40
20
AGE IN WEEKS
CONTROL
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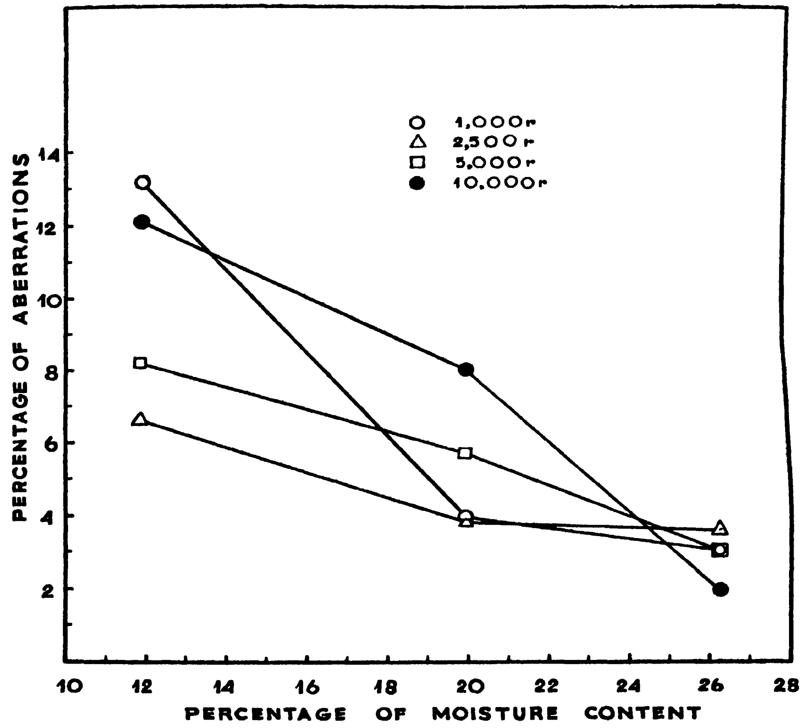
2500 r
5000 r
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

GRAPH 2


○ 1,000r
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PERCENTAGE OF ABERRATIONS
14
12
10
8
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12
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16
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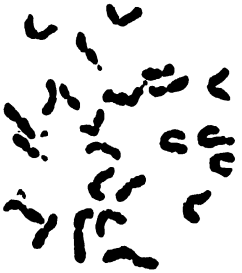
28
PERCENTAGE OF MOISTURE CONTENT



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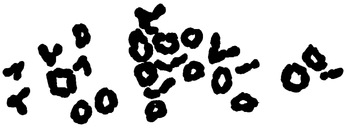

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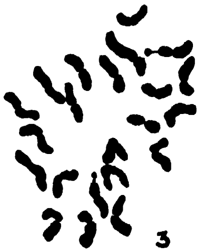

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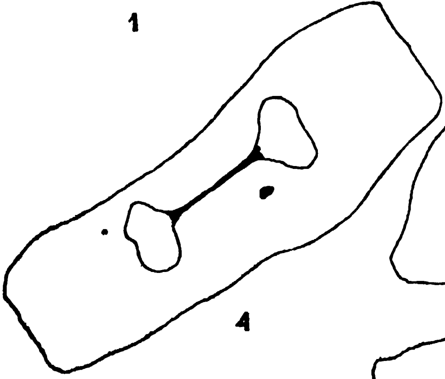

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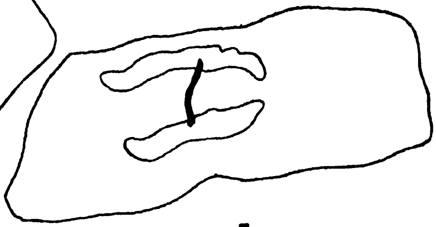

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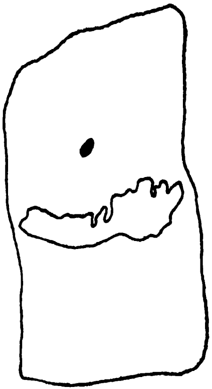

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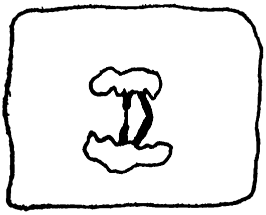

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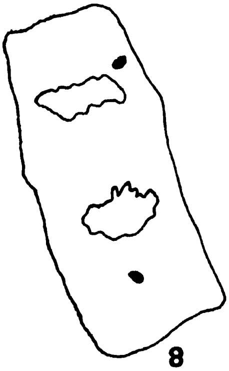

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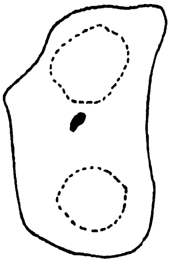

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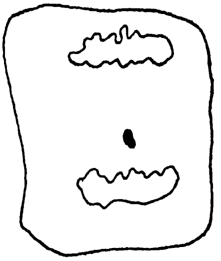

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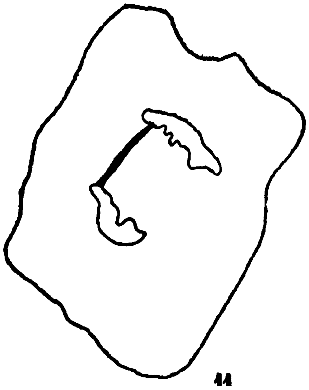

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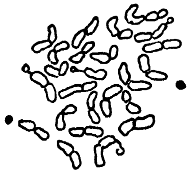

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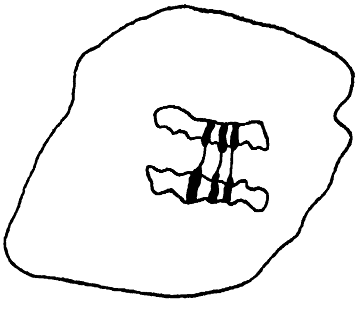

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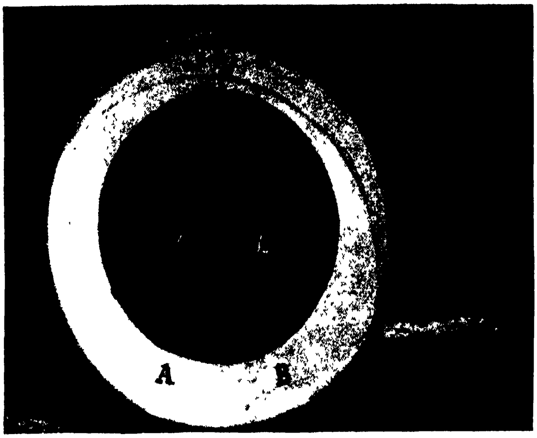

FIG. 14

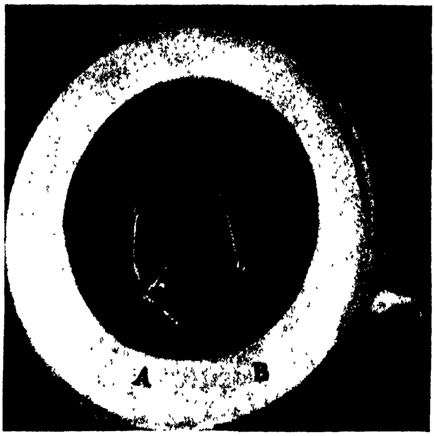

FIG. 15

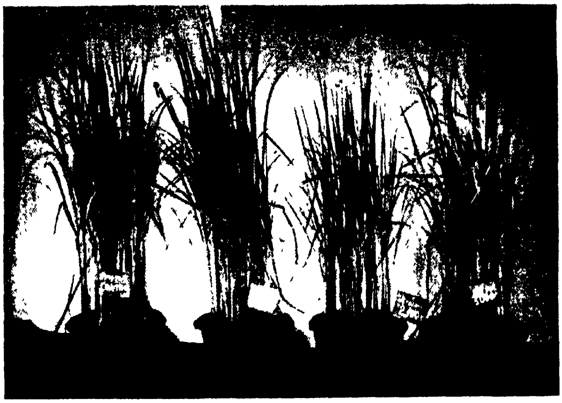

FIG. 16

FIG. 17

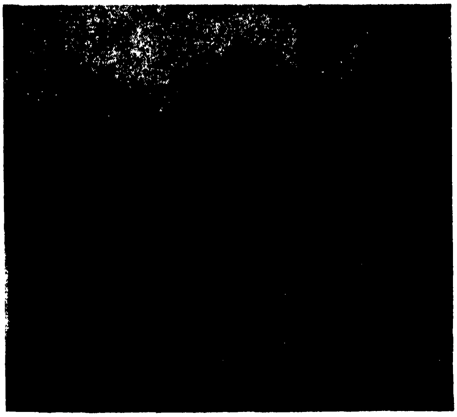

FIG. 18

FIG. 19

VII. LOSS OF TURGOR IN THE PULVINUS OF *MIMOSA PUDICA* DUE TO EXCITATORY CONTRACTION

By B. K. DUTT *and* A. GUHA THAKURTA

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It is generally accepted that the responsive movement of the leaf of Mimosa is associated with a loss of turgor in the irritable cells of the pulvinus. Despite differences in the explanation of the mechanism involved in the process of sap extrusion from the cells, Brucke,[1] Pfeffer,[2] Gardiner,[3] Bose[4] and others agree that the sap from the irritable cells is outflown into the intercellular space and is reabsorbed from there during recovery. Bose has further concluded that the extruded sap is translocated to other directions through the cortex. In arriving at the conclusion regarding the loss of turgidity of the cells previous workers had to depend mostly upon indirect observations. According to Pfeffer contraction and diminution of the volume of pulvinar cells cannot be directly observed. Pfeffer and Bose, however, obtained some experimental evidence of the expulsion of sap from the pulvinar cells due to contraction but detailed studies could not be undertaken by those methods.

Pfeffer separated a leaf-stalk from the pulvinus by sharp cut and kept the plant in moist air. In such condition he observed water to escape through the cut surface of the still attached pulvinus when it had contracted under stimulation. Bose detached a leaf of Mimosa with the pulvinus and placed the sharp-cut end of the pulvinus in a very dilute solution of sodium chloride for several hours. The cut end of the pulvinus was then carefully, without inducing a stimulation, washed with distilled water. Then placing the cut end in a dilute solution of silver nitrate a strong thermal shock was applied to the pulvinus. The contraction of the pulvinus was followed by extrusion of sap from the pulvinus containing sodium chloride which was indicated by a string of white precipitate of silver chloride.

Weintraub[5] has reported that the sections of the pulvinule of Mimosa reopen in water and respond to stimulation. He has observed that during contractile response the vacuoles in the motor cells either disappear or diminish in size and they resume their size on recovery. He has further shown that the pectic material of the motor cell-walls is mostly composed of pectic acid, showing capability of expansion and contraction. His finding suggests that under stimulated condition there is loss of turgidity of the motor cells of the pulvinus with concomitant contraction of the vacuoles, the water being excreted out of the cells as a result of the contraction of the cell-wall.

In a recent publication Mridula Dutt has concluded from anatomical studies that air pressure and not the extrusion of sap plays an important part in the mechanism of response. The excitatory contraction of the pulvinus is brought about by the passage of air from the compressed lower side to the expanded upper side.

In a more recent publication Toriyama has shown that during the contractile movement of the pulvinus potassium salts escape out of the cells into the intercellular spaces. This, he suggests, is brought about by the flow of water from the cells to the intercellular space. He also reports migration of this salt to the transitional tissues situated at the two ends of the pulvinus connected with the stem and the petiole. This indicates that

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there is a flow of sap towards both the stem and the petiole during the contractile movement. In a previous communication he has reported that there are large intercellular spaces in the petiole which open into the intercellular spaces of the pulvinus through the transitional tissues.

In the present communication an experimental device is described by which the expulsion of water from the pulvinus of *Mimosa pudica,* due to its contractile movement, can not only be directly observed but it can also be quantitatively measured. The investigation has revealed that the outflowing sap from the pulvinus can move through the petiole and the stem, and it can also escape through the epidermal outlets.

METHOD OF EXPERIMENT

The observation is made with the help of a micro-potometer consisting of an L-shaped glass tube, the horizontal arm of which is a capillary tube having a cross-section 0·04 sq. mm. A small stem piece with a leaf is detached from the plant and the stem is introduced into the short wide vertical arm of the potometer containing water, and sealed watertight with modelling clay. In selecting specimens, active leaf from the hard wood portion of the stem is taken. Generally the fourth or the fifth leaf from the top is selected, which is found to yield the best result. For eliminating transpiration the leaflets with a portion of the petiole are discarded, and the upper cut end of the stem and the cut end of the petiole are sealed with collodion flexile. As there is considerable transpiration even in such condition the plant material with a portion of the potometer is enclosed in a thoroughly moist glass chamber, the open arm of the potometer being allowed to protrude out for observation of the displacement of the water meniscus. The displacement of the meniscus is followed with a micrometer screw of a travelling microscope which is connected with a writing lever of a recorder for obtaining a magnified record of the expelled sap due to pulvinar contraction. The petiole is connected with a second lever of the recorder with a silk thread passing through a small hole on the top of the moist chamber, for recording the mechanical response of the pulvinus. For introducing excitatory stimulation to the pulvinus, mechanical or electrical stimuli can be applied from outside the chamber by suitable means. The minimum electric shock required has been found to be ~2 volts produced by an induction coil. The whole experimental device is represented diagrammatically in Fig. 1.

A specimen record representing the outflow and inflow of water with contraction and recovery and the record of the responsive movement of the pulvinus simultaneously taken is given in Fig. 2. The initial portion of the upper curve, which represents suction rate, shows that in spite of thoroughly moist condition of the chamber suction persisted at a very slow rate. In computing values for the suctional changes due to responsive movement of the pulvinus from the curves this residual suction rate was taken as the base line. In some specimens, however, the initial suction rate was found to be nil.

The outflow of sap is represented by an up curve and the inflow of sap by a down curve. The volume of displaced water from the pulvinus is determined from the increase in length of the column of water and the cross-section of the capillary tube. In the experiment of which the record is shown in Fig. 2, the volume of water expelled during contraction and reabsorbed during recovery has been found to be 0·0192 and 0·036 c.mm. respectively.

The quantity of water expelled during contraction has been found to be widely variable in different specimens. This is shown in Table I. In repeated excitatory contraction of the same pulvinus the difference in the quantity of expelled water has, however,

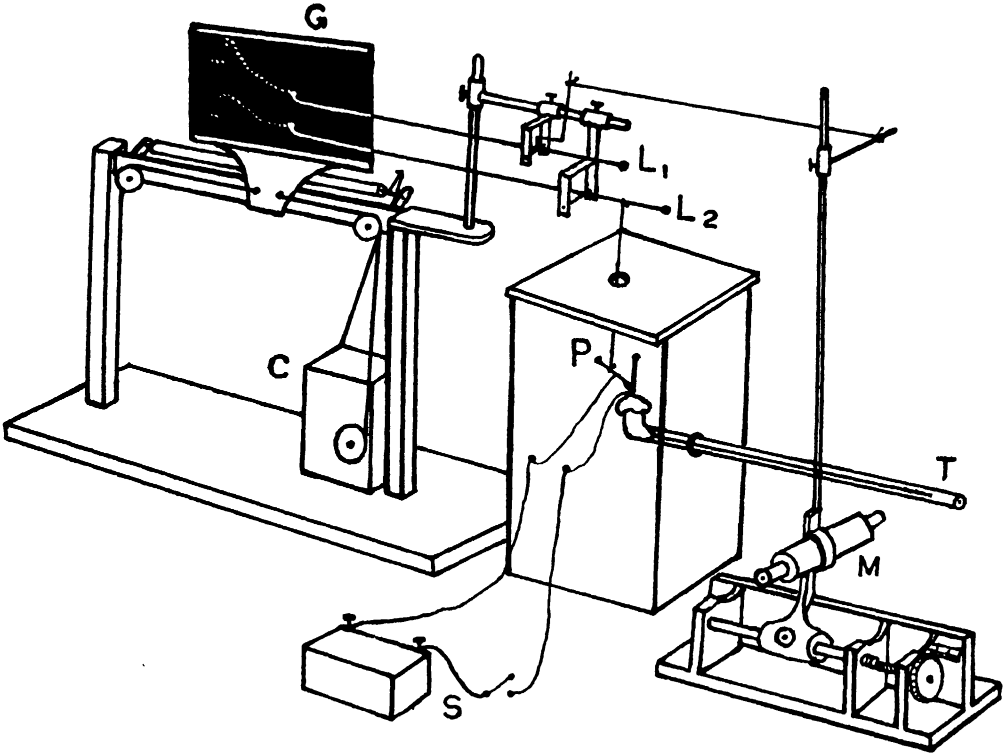

FIG. 1. Diagrammatic representation of experimental arrangement for recording expulsion of sap from the pulvinus of *Mimosa pudica* due to excitatory contraction.

P, plant mounted in the capillary potometer *T*; L_2, recording lever attached to the petiole of the plant; *M*, travelling microscope with micrometer screw attached to the lever L_1; *S*, shocking arrangement for stimulating the plant; *G*, smoked glass plate; *C*, clock-work arrangement; moist chamber is represented in dotted lines.

been found to be very small, and in some cases almost negligible. The quantity of water reabsorbed during recovery of the pulvinus has been found to greatly exceed the quantity expelled during contraction. It will be evident from the table that the excess water absorbed during recovery varies between 50 to 760% of the expelled water in different specimens. This indicates that the total quantity of water expelled out of the pulvinus during contraction is not wholly ejected through the cut end attached to the potometer. A portion only flows through the potometer and the rest probably through epidermal outlets. In regaining the loss of turgor in the pulvinus during recovery the entire quantity is reabsorbed from the potometer, and this is the cause of greater absorption of water during recovery. The wide variation of values in different specimens can be attributed to both physical and physiological variations in individual ones. This view is further supported from the fact that in the case of young and tender specimens the difference between the two values becomes much greater.

During vigorous growth of the plant a portion of apical region of the stem remains soft and succulent. In selecting young specimens, second or third leaf from the top, growing in this region were taken. In these specimens it was found that the quantity of water expelled under stimulation, through the potometer, was much lower than that obtained with fully mature leaves in previous experiments, and the difference between the expulsion and reabsorption of water much larger. This is shown in Table II. It will be found from the table that quantity of water absorbed in recovery in these specimens exceeds the value of expulsion by 733 to 1,240%. This indicates that in the young specimens the expelled sap receives much less resistance to escape through the epidermal outlets than through the cut surface.

TABLE I

Showing expulsion of sap through the lower cut end of the stem of Mimosa due to excitatory contraction of the pulvinus (mature leaf)

No. of experiment	Water expelled through potometer in c.mm.	Water reabsorbed during recovery in c.mm.	Excess of reabsorption over expulsion in per cent
1	0·0208	0·032	54
2	0 0162	0 0272	62
3	0·0256	0·054	112
4	0·05	0·2	300
5	0·04	0·16	300
6	0·0152	0·0336	121
7	0·019	0·036	87
8	0·07	0·26	271
9	0·05	0·2	300
10	0·008	0·0296	270
11	0·0096	0·032	233
12	0·0088	0·0272	209
13	0·027	0·056	105
14	0·019	0·054	184
15	0·004	0·0344	760
16	0·0064	0·04	525
17	0·05	0·19	280
18	0·04	0·22	450
19	0·016	0·024	50
20	0·0176	0·028	64

It has already been mentioned that the plant material used in the experiment has three cut surfaces, the lower and the upper cut ends of the stem and the cut end of the petiole. So far, the expulsion of sap through the lower cut end of the stem has been described. Next, the outflow of sap through the other two cut ends has been studied individually.

In studying the outflow of sap through the upper cut end of the stem the wider end of the potometer is directed downward, so that the plant remains oriented in its natural position. In observing the outflow through the cut end of the petiole the bent end of the potometer has to be placed almost horizontally.

TABLE II

Showing expulsion of sap through the lower cut end of the stem of Mimosa due to excitatory contraction of the pulvinus (young leaf)

No. of experiment	Water expelled through potometer in c.mm.	Water reabsorbed during recovery in c.mm.	Excess of reabsorption over expulsion in per cent
1	0·0034	0·041	1,200
2	0·0048	0·043	800
3	0·0064	0·072	1,025
4	0·0056	0·058	900
5	0·0088	0·108	1,127
6	0·0096	0·102	966
7	0·0048	0·04	733
8	0·004	0·053	1,240

The quantity of outflow through the upper cut end of the stem is very small in comparison to the quantity absorbed in recovery, the difference obtained varying from 380 to 2,075%. This indicates that greater resistance is encountered in outflowing the sap through the upper cut end of the stem and as a result the major quantity escapes through epidermal outlets of the plant. The results are summarized in Table III.

TABLE III

Showing expulsion of sap through the upper cut end of the stem of Mimosa due to excitatory contraction of the pulvinus (mature leaf)

No. of experiment	Water expelled through potometer in c.mm.	Water reabsorbed during recovery in c.mm.	Excess of reabsorption over expulsion in per cent
1	0·0016	0·0112	600
2	0·0016	0·0216	1,250
3	0·007	0·043	500
4	0·008	0·038	380
5	0·004	0·072	1,700
6	0·0048	0·068	1,316
7	0·0016	0·02	1,150
8	0·0016	0·012	650
9	0·0032	0·069	2,075
10	0·004	0·063	1,480
11	0·0048	0·06	1,100
12	0·0064	0·0616	925

Greatest outflow has been recorded through the cut end of petiole. The quantity of absorbed sap in recovery is greater only by 5 to 80% than the quantity of outflow through the potometer during contraction. The results of the experiments are given in Table IV. From comparison of the quantitative values obtained with the three outlets it can be definitely stated that the outflow through the cut end of the petiole encounters the least resistance.

TABLE IV

Showing expulsion of sap through the petiolar cut end of Mimosa due to excitatory contraction of the pulvinus (mature leaf)

No. of experiment	Water expelled through potometer in c.mm.	Water reabsorbed during recovery in c.mm.	Excess of reabsorption over expulsion in per cent
1	0·21	0·22	5
2	0·21	0·26	24
3	0·09	0·1	11
4	0·08	0·1	25
5	0·04	0·07	75
6	0·06	0·1	66
7	0·09	0·11	22
8	0·11	0·12	9
9	0·06	0·1	67
10	0·08	0·09	12
11	0·1	0·18	80
12	0·11	0·15	36
13	0·06	0·09	50
14	0·07	0·08	14

EXPERIMENT WITH *Desmodium gyrans*

In order to study whether the autonomous motility of the pulvinus also brings about a change in its turgidity, similar experiments were undertaken with Desmodium leaflet. For observation of the variation of suction in Desmodium during its movement a finer potometer tube having a cross-section of 0·03 sq. mm. was used. A motile leaflet attached to a portion of the petiole was mounted in water-tight condition in the wide end of the potometer. A portion of the potometer with the leaflet was enclosed in a moist chamber similarly as was done in the experiment with Mimosa. The free moving leaflet was attached to one of the recording levers with a single strand cocoon thread passed through a small hole on the upper side of the moist chamber for recording the autonomous movement. The movement of the water meniscus in the potometer was simultaneously recorded by the second lever.

A number of experiments were performed with the above method. In all of them the normal residual rate of suction was found to be quite independent of the autonomous movement. No change was observed in the suction rate by the rise and fall of the leaf. A typical record is given in Fig. 3.

It can be noted in this connection that the rise and fall of the leaflet of *Desmodium* is a comparatively slow and gradual process, whereas the contractile movement of the pulvinus of Mimosa occurs instantaneously. Moreover the autonomous movement of Desmodium is not a simple up and down linear movement, the pathway described is more or less an ellipse[7] indicating propagation of alternate contraction and expansion in a circular direction round the pulvinus.

DISCUSSION

The present experiment with pulvinar petiolar preparation of Mimosa has shown that water is expelled through the adjoining tissues of the pulvinus with the responsive movement of the leaf. If the conclusion of Mridula Dutt, that the responsive movement of the pulvinus is brought about by the transference of trapped air from the abaxial side to the adaxial side of the pulvinus, is correct there would be no possibility for such expulsion of water from the adjoining tissues coinciding with the movement of the pulvinus. Because in that condition, the total volume of the pulvinus would not change, the contraction of the lower side of the pulvinus being only compensated by an equivalent expansion of the upper side and consequently the hydrostatic condition of the adjoining tissues would remain unaffected. This is not in conformity with observations made by previous workers. Pfeffer showed by micrometric measurement that in the responsive movement of the pulvinus the volume of the lower half is much reduced and that of the upper half remains almost unchanged. Bose by measuring the diameter of the pulvinus with Sphygmograph found that the total volume of the pulvinus is much reduced during the responsive movement. They have further suggested that with the contraction of the pulvinus water is expelled from it and conducted through the adjoining tissues of the stem and the petiole. The findings of Toriyama which suggests the expulsion of potassium salts from the cells to the intercellular spaces and then towards the transitional tissues during contractile movement, are also in conformity with the above view. The present work also fully supports this view with further experimental evidences. The flowing out of water from the cut surface attached to the potometer was, no doubt, caused by the increase of turgor pressure of the adjoining tissues by the expulsion of water from the pulvinus during contraction. The specimen was enclosed in a moist chamber and it was allowed to absorb water by one of its cut surfaces, the other two cut ends being sealed. Thus the tissue being in a saturated condition it is conceivable that it cannot retain more water. With further increase of hydrostatic pressure in that condition the additional water will be forced out.

If a quantity of water expelled through the potometer during the contraction of the pulvinus had been equal to the quantity absorbed during expansion of the pulvinus in recovery, it could be taken as the actual quantity of expulsion from the pulvinus. But the wide difference between the two values indicates that a part of the total quantity of water expelled from the pulvinus has been ejected through the potometer and the rest has escaped through the epidermis. During recovery the epidermal openings presumably close up and the total quantity of water lost through the epidermis and through the cut surface has to be supplied from the potometer tube and hence the value has become higher. The quantity of water absorbed in recovery to gain the normal position of the pulvinus, therefore, can be taken as the index for the actual quantity of water expelled from the pulvinus during contraction.

The quantity of outflow through the cut surface has been shown to depend upon different factors. In younger specimens the proportionate outflow through the cut surface is much lower than that of the mature one. It shows that the flowing out of water through the epidermis of young specimens encounters lesser resistance. Likewise the proportionate outflow through the upper cut surface of the stem has been found to be comparatively much less than that from the lower cut surface. On the other hand when the petiolar cut surface is enclosed in the potometer the major outflow of the expelled water has been through it and a small portion through the epidermis. The presence of large intercellular spaces in the petiole, as reported by Toriyama, may be the cause of easy flow of sap through the petiolar tissue. The results of these experiments indicate that the flowing out of the expelled water through the adjoining tissues is controlled by the internal tissue resistance; it flows through channels where it receives less resistance. It can, therefore, be reasonably assumed that in intact plant the water expelled from the contracted pulvinus escapes through the epidermal outlets or is retained in the adjoining tissues if they are not fully turgid, to be reabsorbed from there in recovery.

SUMMARY

A micro-potometer has been described with which the expulsion of sap from the pulvinus of Mimosa due to excitatory contraction can be studied.

The experiment was conducted with a stem piece having a pulvinus and a portion of petiole attached to it.

When the lower cut end of the stem was introduced into the potometer, the upper cut end of the stem and the cut end of the petiole being sealed, a quantity of water flowed out into the potometer during the contractile movement of the pulvinus; during recovery of the pulvinus, a greater quantity of water was reabsorbed from the potometer. In younger specimens the difference between the quantity of expulsion and reabsorption was larger.

When the upper cut end was connected to the potometer the quantity of water expelled was less than that observed in the case of lower cut end, and the difference between expulsion and reabsorption was very high.

In the case of petiolar cut end quantity of water expelled through the potometer was the highest.

From greater intake of water in recovery it has been concluded that the sap dislodged from the pulvinus partially outflows into the potometer, the rest escapes through the epidermal outlets; the quantity absorbed in recovery represents the total expulsion of sap from the pulvinus during contraction. It has further been concluded from the difference in outflow through different cut ends, that the volume of outflow through adjoining tissues is controlled by the internal resistance.

Similar experiments were conducted with Desmodium leaflet but no change in the rate of suction was observed with the autonomous movements of the leaf.

ACKNOWLEDGEMENT

Grateful thanks are due to Dr. D. M. Bose, Director of the Institute, for his keen interest in this work and for his valuable suggestions.

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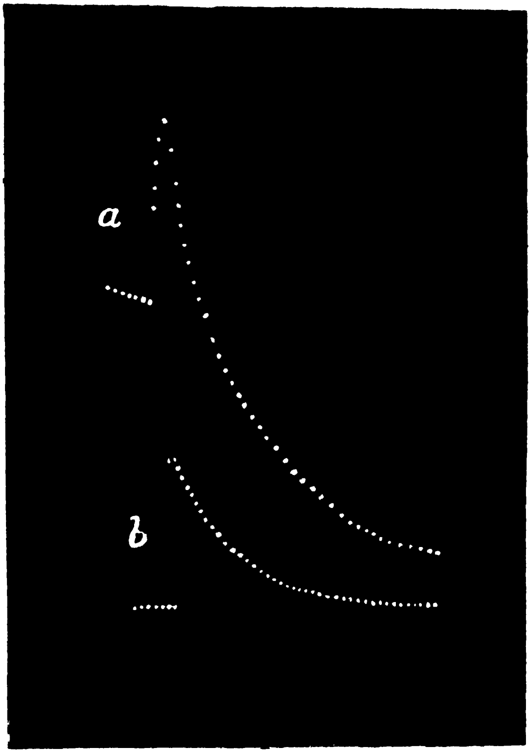

FIG. 2. Record showing expulsion of sap from the pulvinus of Mimosa due to its contractile response.

Curve *a*, showing expulsion of sap by up curve and subsequent recovery of suction rate by down curve ; note absorption of greater quantity of water during recovery.

Curve *b*, showing contractile response and recovery by up and down movements respectively.

Dots at intervals of 24 secs.

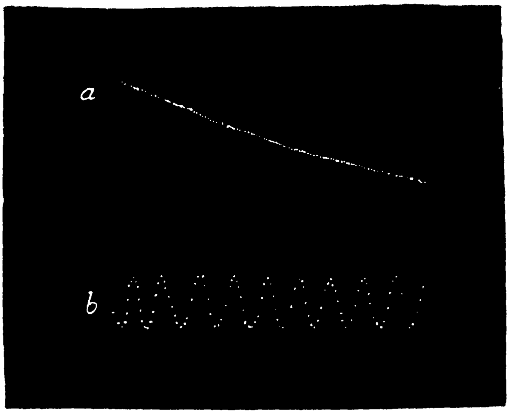

FIG. 3. Record showing no change in the suction rate of the leaflet of *Desmodium gyrans* during its autonomous movements ; *a*, residual suction rate (down curve) in moist chamber ; *b*, autonomous rhythmic pulsation.

Dots at intervals of 12 secs.

VIII. CHEMICAL INVESTIGATION OF INDIAN SPICES. PART I

By SACHINDRA K. CHAKRABORTI, *Bose Institute, Calcutta-9*

Indian spices have been widely used from ancient times not only as condiments and colouring agents in foodstuff but also for their therapeutic and physiological actions[1, 2]. Although much work has been done on the volatile constituents of the spices, little attention seems to have been paid to the non-volatile matters. Moreover, since some of the plant pigments have been found to possess physiological properties, it is desirable to investigate the nature of the pigments, if any, present in the spices. A systematic chemical examination of the non-volatile matters of Indian spices has, therefore, been taken up.

In the present communication the results of investigation of five common spices are described. These are: *Cuminum cyminum* Linn., *Carum copticum* Benth. and Hook., *Carum carvi* Linn., *Coriandrum sativum* Linn., and *Foeniculum vulgare* Gaertn., all belonging to the N.O. Umbelliferae. The materials for this work were obtained from the local market.

General procedure.—The air-dried crushed materials were successively extracted with petroleum ether (b.p. 60–80°), ether and rectified spirit or directly with rectified spirit. Petroleum ether and ether extract residues were separately given a dilute alkali wash and the neutral fractions chromatographed on Brockmann's alumina. The alkali soluble portions were acidified and the residues, if any, tested for flavonoid constituents. The alcoholic extracts were treated successively with neutral and basic lead acetates by usual procedure, and the lead salts were then decomposed. The resulting materials were then purified.

The alcoholic extract of the air-dried seeds of *C. cyminum* (commonly known as *zira*) yielded two flavone-glycosides (I and II) and *d*-mannitol (III). The glycoside (I) had an indefinite m.p. of 225–40° and could not be characterized. The crystalline aglycone, m.p. 315–18° (decomp.), obtained by acid hydrolysis of this glycoside, was found to be identical with luteolin[3] (5: 7: 3′: 4′-tetrahydroxyflavone). The glycoside (II), fine needles, m.p. 228–32°, has been identified as apiin[4] (apigenin-7-glycoside) and its aglycone, m.p. 340–45°, as apigenin (5: 7: 4′-trihydroxyflavone). It is interesting to note that here is another instance of occurrence of these flavones as their glycosides in pair.[5] No hesperidin could be isolated from this source as reported by G. Klein.[6] No basic substances could be detected in the spice.

The seeds of *C. copticum* (also known as *ajowan*) have been found to contain a yellow crystalline flavone, m.p. 291–94°, but the yield was insufficient to permit further work. The neutral fraction gave a steroidal substance, m.p. 140–50°, in low yield.

The seeds of *C. carvi* (commonly known as *shahjira*), which is closely allied to *C. cyminum*, was found to contain a hydrocarbon, m.p. 62-63°, and a neutral substance, m.p. 80–82°. No flavonoid could be isolated.

The root and stem of *C. sativum* (known as *dhanay*) yielded a steroidal compound, m.p. 136–38° (β-sitosterol) and a neutral component, m.p. 78–83°, both in very low yield.

F. vulgare or fennel (known locally also as *mouri*) fruits were found to contain a colourless product, m.p. 270–80°, in the ether extract. It shows a positive Liebermann-Burchardt test. The alcoholic extract of the fruits showed the presence of a flavonoid compound.

EXPERIMENTAL

(The melting point recorded in this paper are uncorrected and are determined in H_2SO_4 bath unless otherwise mentioned. Light petroleum had b.p. 40–60°. Homogeneity of the compounds was established by paper chromatography. The samples were dried *in vacuo* over P_2O_5 before analysis.)

Cuminum cyminum: Isolation of glycoside (*I*).—The air-dried powdered seeds of *C. cyminum* (1,800 g.) were continuously extracted with petroleum ether (b.p. 60–80°) for 20 hours to remove most of the essential oils, fatty matter, etc. The defatted material was then refluxed with rectified spirit (1·5 l.) for 5 hours and filtered. This process was repeated three times with fresh rectified spirit. The combined extract was concentrated to 900 c.c. under reduced pressure and treated with an excess of neutral lead acetate. The bulky deep yellow precipitate of the lead salt was filtered off and the filtrate marked (A). The lead salt was thoroughly washed with hot alcohol and the washing added to the filtrate (A). A suspension of the lead salt in 50% hot alcohol was decomposed with hydrogen sulphide, the lead sulphide filtered off and the precipitate washed with alcohol. The filtrate was concentrated to 100 c.c. and left in the refrigerator overnight when a yellow substance with plenty of gum separated. The mother-liquor on keeping for about a week in the cold gave a second crop of the colouring matter. The crude substance on crystallizing from boiling water yielded a yellow amorphous solid (0·8 g.; yield 0·044%).

Isolation of glycoside (*II*).—The hot filtrate (A), on treatment with excess basic lead acetate and on subsequent decomposition of the salt in the manner described above, gave a thick gelatinous deposit of glycoside (II). A second crop was obtained on concentration of the mother-liquor. The crude product was washed with dilute alcohol to remove tarry matters and crystallized from boiling water, when a pale yellow solid was obtained (4·3 g.; yield 0·24%).

The dark red-coloured mother-liquor, left after separation of (II), was concentrated to 100 c.c. and left in the refrigerator for a couple of days. A gummy material was deposited, which on washing with methanol gave colourless needles (yield 0·22%). Purified by repeated crystallization from aqueous alcohol, it had the m.p. 165°. The mixed m.p. of this substance with an authentic sample of *d*-mannitol recorded no depression. The identity was confirmed by preparing the hexa-acetyl derivative, m.p. 121°, by the usual method, and comparing it with hexa-acetyl-*d*-mannitol.

Hydrolysis of (*I*): *Isolation of luteolin.*—The glycoside (I) could not be obtained pure by crystallizations and its m.p. was not sharp. Attempt to purify it by repeated adsorption over celite-charcoal mixture (9: 1) and elution according to the method of T. Swain [7] was also unsuccessful. The substance (0·6 g.) was hydrolysed with 7% alcoholic H_2SO_4 (20 c.c.) under reflux for 8 hours. The product was worked up as usual and the aglycone filtered. The filtrate (after neutralization with $BaCO_3$) reduced Fehling's solution. The aglycone crystallized in golden yellow rectangular plates from dilute alcohol (1: 1), m.p. 315–18° (decomp.). On repeated crystallizations from aqueous ethanol (1: 1), acetone or acetone-benzene mixture, the m.p. remained unchanged. It exhibited all the characteristic colour reactions of a hydroxy flavone; deep violet colour with $FeCl_3$, red-orange colour when reduced by Mg and HCl; intense yellow colour with dilute alkali. An authentic sample of luteolin recorded no depression in m.p. when mixed with our product and their R_f values were also same (Table II). (Found: C, 63·15; H, 3·84. Calc. for $C_{15}H_{10}O_6$: C, 62·92; H, 3·52 per cent.).

Luteolin tetra-acetate.—A mixture of the aglycone of (I) (0·1 g.), acetic anhydride and pyridine on gently heating for 3 hours gave a product (0·12 g.) which was crystallized repeatedly from benzene as colourless needles, m.p. 226–28°. (Found: C, 60·81; H, 4·06. Calc. for $C_{23}H_{18}O_{10}$: C, 60·77; H, 3·99 per cent.).

In the literature [4, 8, 12] different m.p.'s have been recorded for the tetra-acetate of luteolin, namely 222–24°, 214-15°, 226-27°.

Luteolin trimethyl ether.—A suspension of the aglycone of (I) (0·15 g.) in ether was mixed with an excess of ethereal diazomethane and kept at 0° for 24 hours. Evaporation of the solvent and crystallization of the residue (0·1 g.) from benzene-light petroleum gave the methyl ether as yellow needles, m.p. 167-68°. Luteolin trimethyl ether has been reported [8] to melt at 161–63°. Though insoluble in dilute alkali, its alcoholic solution gave a violet colour with $FeCl_3$. (Found: C, 65·77; H, 5·02. Calc. for $C_{18}H_{16}O_6$: C, 65·83; H, 4·91 per cent.).

Glycoside (II).—The glycoside (II) containing much of gummy impurities could not be purified easily. The m.p. of the substance, on repeated crystallizations from aqueous alcohol, was considerably lowered and was not sharp. However, a number of crystallizations from boiling water eventually yielded very fine colourless needles of m.p. 228–32°. Apiin isolated from Parsley seeds [4] melted at 230–32° (m.p. reported by von Gerichten,[9] 228°). The R_f values of the glycoside (II) and of apiin determined under identical conditions were the same.

Hydrolysis of glycoside (II): Isolation of apigenin.—The glycoside (2 g.) was hydrolysed in 7% alcoholic H_2SO_4 (100 c.c.) for 8 hours and the aglycone was isolated as usual. Sparingly soluble in most of the organic solvents excepting pyridine, it was crystallized repeatedly from ethanol, m.p. 326–32°. From aqueous pyridine fine yellow needles were obtained, m.p. 340–45° (Kofler block), the reported [4, 8, 10, 11] m.p.'s being 343–45°, 343-44°, 338-44°, 347-48°. The m.p. did not change on further crystallization. (Found: C, 66·61; H, 4·11. Calc. for $C_{15}H_{10}O_5$: C, 66·64; H, 3·73 per cent.).

The sugar component.—The filtrate left after separation of apigenin was neutralized by means of barium carbonate and the clear solution was evaporated to dryness. The residue was then taken up in pyridine and subjected to paper chromatography by the standard method. The results (Table I) show that the sugar components are glucose and apiose.

TABLE I

R_f values of sugar components of glycoside (II)

Solvent	Sugar components of glycoside (II)		Sugar components of apiin		*d*-Glucose
	1	2	Apiose	Glucose	
80% *ter*-BuOH containing 0·25 g. picric acid at 33°	0·70	0·66	0·71	0·65	0·66
Phenol saturated with water at 29° ..	..	0·41	..	..	0·41
BuOH: pyridine: water (10: 3: 3) ..	0·55	0·23	..	..	0·23

Apigenin triacetate.—Apigenin (0·2 g. from glycoside II) formed, on treatment with pyridine-acetic anhydride, a triacetate (0·19 g.) as colourless needles (from benzene-light petroleum mixture), m.p. 182-83°, which agreed with that of apigenin triacetate.[8] (Found: C, 63·89; H, 4·14. Calc. for $C_{21}H_{16}O_8$: C, 63·66; H, 4·07 per cent.).

Dimethyl ether of apigenin.—Apigenin (0·15 g. from glycoside II) was methylated with excess ethereal diazomethane and the cream-coloured residue (0·09 g.) was worked up as in the case of luteolin. Bright yellow rectangular plates, m.p. 173-74°, were obtained from benzene-light petroleum. (Found: C, 68·42; H, 4·56. Calc. for $C_{17}H_{14}O_5$: C, 68·43; H, 4·73 per cent.). The m.p.'s of apigenin dimethyl ether recorded in the literature[10,12,13] are 168–69°, 171-72°, 170-71°.

The above methyl ether (50 mg.) was acetylated by the usual method. A colourless crystalline product, m.p. 200-201°, was obtained. (Found: C, 67·24; H, 4·93. Calc. for $C_{19}H_{16}O_6$: C, 67·03; H, 4·74 per cent.). The acetyl methyl ether of apigenin is reported[13] to melt at 199-200°.

Paper chromatography of the flavonoid constituents.—The flavonoids (I) and (II), as also their aglycones, were chromatographed on paper using two solvent systems. All the four compounds were co-chromatographed and the mixture separated well. Identical R_f values were obtained when these were chromatographed side by side with specimens of apiin, apigenin and luteolin, under similar condition (Table II).

TABLE II

Rf values of the flavonoids

Substance	Phenol saturated with water at 32°	BuOH: HAc: H_2O (4: 1: 1) at 34°
Glycoside (I) ..	0·68*	0·25†
Glycoside (II) ..	0·82	0·55
Aglycone of (I) ..	0·73	0·74
Aglycone of (II) ..	0·89	0·84
Apiin ..	0·82	0·52
Apigenin ..	0·89	0·84
Luteolin ..	0·72	0·75

* at 28°; † at 29°.

Carum copticum.—The air-dried coarsely powdered seeds (850 g.), after defatting with petroleum ether (b.p. 60–80°), were refluxed thrice with boiling rectified spirit (700 c.c.) each time for 6 hours and filtered. The total extract (2·1 l.) was reduced to half the volume by distillation under reduced pressure and treated successively with neutral and basic lead acetate as described before. The neutral lead acetate precipitate on decomposition gave no crystalline residue. The basic lead acetate precipitate was decomposed and the crude pigment (0·085 g.) separated as a brown solid from the concentrated solution after seven days. It was repeatedly crystallized from aqueous ethanol and aqueous pyridine when the flavone was obtained as constant-melting bright yellow rectangular rods, m.p. 291–94°. Insoluble in petroleum ether and benzene, it is readily soluble in ethyl acetate, ethyl and methyl alcohol and pyridine. Moderately soluble in ether, it gave an intense yellow coloration with aqueous alkali. An alcoholic solution of this substance on reduction with magnesium and hydrochloric acid turned orange-red. With alcoholic ferric chloride, it produced an olive-green colour. It has a R_f value of 0·31 in phenol saturated with water at 32° and 0·62 in butanol-acetic-acid-water (4: 1: 1) at 34°.

The residue from the petroleum ether was taken up in ether, washed with alkali, dried over sodium sulphate and the solvent removed. The alkali soluble portion was acidified and found to contain no crystalline matter. The residue obtained by removing ether was chromatographed over Brockmann's alumina and eluted with solvents of increasing polarity. Elution with light petroleum ether (300 c.c.) afforded a colourless crystalline solid. Subsequent elution with other solvents and evaporation left no residue. The solid was re-chromatographed and crystallized from aqueous ethanol in colourless flakes, m.p. 140–50°. It is soluble in petroleum ether, ether, chloroform, benzene, acetone and ethanol. It shows a positive Liebermann-Burchardt reaction (purple→blue→green).

Carum carvi.—Powdered seeds (925 g.) of *C. carvi* were extracted with rectified spirit as in the previous case, and the combined extract (2·1 l.) on concentration deposited a colourless waxy solid (1 g.). The substance was dissolved in benzene-petroleum ether mixture (25 c.c.) and was chromatographed over alumina. Elution with light petroleum ether (200 c.c.) gave colourless glistening crystals, m.p. 62-63°, and elution with benzene (100 c.c.) gave a tiny amount of neutral residue, m.p. 80–82°. The former was obtained as a constant-melting compound from benzene-acetone mixture. (Found: C, 84·85; H, 14·83 per cent.)

Coriandrum sativum.—The crushed root and stem of the plant (120 g.) were extracted with ether in a Soxhlet for 20 hours. The residue was divided into neutral and alkali-soluble fractions. The alkali-soluble fraction on acidification yielded no crystallizable residue. The neutral fraction on columnar chromatography over alumina and elution with benzene (140 c.c.) gave a neutral substance, m.p. 78–83°, and elution with benzene-ether (1: 1) (120 c.c.) yielded colourless flakes, m.p. 125–30°. The latter responds to Liebermann-Burchardt reaction (red→violet→blue→green). It was crystallized from aqueous ethanol as colourless flakes, m.p. 136–38°, undepressed on admixture with an authentic sample of β-sitosterol.

Foeniculum vulgare.—Powdered fruits (445 g.) were extracted successively with petroleum ether (b.p. 60–80°) and ether for 20 and 25 hours respectively. No crystalline compounds could be obtained on working up the petroleum ether extract. The ether extract on concentration deposited a colourless mass in the cold (yield 0·007%). It was crystallized first from aqueous ethanol and then from pyridine, m.p. 270–80°. Insoluble in petroleum ether and sparingly soluble in ether, it is soluble in benzene, ethanol and pyridine. It shows Liebermann-Burchardt reaction (violet→blue→green). The fruits were then refluxed with rectified spirit for 5 hours and filtered. The process was repeated thrice with fresh solvent. The total extract (1·5 l.) was worked up as described in the general procedure. The concentrated alcoholic extract gave a faint orange-red colour on reduction with magnesium-hydrochloric acid but no flavonoid compound could be isolated.

SUMMARY

Chemical investigation of a number of Indian spices has been carried out. Two flavone-glycosides and *d*-mannitol have been isolated from the seeds of *Cuminum cyminum*. One of the glycosides has been identified as apiin (the corresponding aglycone as apigenin) and the aglycone of the other as luteolin. The seeds of *Carum copticum* contain a flavone and a steroidal substance in low amounts. A hydrocarbon and a neutral substance were isolated from the seeds of *Carium carvi.* The root and stem of *Coriandrum sativum* yielded β-sitosterol and a neutral substance. *Foeniculum vulgare* (fruit) contains a neutral substance of steroidal nature.

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IX. ABSOLUTE DIFFERENTIAL RANGE-SPECTRUM OF SEA-LEVEL MUONS AT 12° N.

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INTRODUCTION

Cosmic-ray μ-meson intensity has been measured by a number of investigators at sea-level mainly at latitudes between 45° N. and 50° N. The techniques employed for this measurement in most cases utilize either (1) the non-showering property of the muons as compared to an electron of same energy or (2) its instability due to which it decays into an electron with a mean life of 2·15 micro-seconds. The chief difficulty of the first method lies in the fact that it is not possible to distinguish between a muon and an electron by its non-showering property alone when the absorber used to stop the meson is so small that it allows low energy electron to pass through without showering. To avoid this difficulty one or even two cloud chambers (Lichtenstein, 1954) have been used to determine the mass of the particle by residual-range momentum relationships. The use of a magnetic field, however, sets a lower limit of 250 Mev./c to the momentum of the muons investigated. But the change in the ionization produced by a muon in passing through a number of metal plates of suitable thickness placed inside a cloud chamber is almost always sufficient to distinguish it from either a proton or an electron, and this property can be used to recognize positive and negative muons with equal efficiency.

All methods using the unstable nature of the muon suffer from a large number of uncertainties (D. Brini *et al.*, 1955) corrections for which are difficult to evaluate. The principal sources of error of this method are the detection inefficiency of the μ-e decay due to a broad spectrum of the electron secondaries (10–55 Mev.) and the high capture probability of negative muons which very scarcely give rise to decay electrons even in elements of medium atomic number. This necessitates a correction involving the ratio of positive to negative muons in the sea-level spectrum which is still not accurately known. There is a serious source of error common to all determinations of the spectrum of low energy muons and this is the complete loss of a high percentage of the particles when an absorber is placed somewhere in the detecting apparatus to shift the spectrum investigated to higher values. This has been shown by Germain (1950) to be as high as 50% when the absorber above his apparatus was 15 inches of lead. It seems that the importance of this 'scattering out' of the particles has not been taken carefully into consideration except by York (1952) who, however, has made a mistake in evaluating the magnitude of the electron contribution to the muon spectrum, as has been pointed out by Lichtenstein (1954). The recent determination of the muon spectrum by the delayed coincidence method using a pair of scintillation counters by Brini *et al.* (1955) has, however, taken into account the loss of particles due to the interposition of the absorber in between the scintillators by a complicated and elaborate process. Fafarman's (1956) determination of the muon intensity by means of a plastic scintillator which serves both as an absorber and detector seems to be the most accurate in the lowest energy interval. Whatever be the corrections and uncertainties in the various methods adopted up to date for the measurement of the muon flux, one thing stands out very prominently that the value obtained for this flux varies from 2×10^{-6} to $8{\cdot}4 \times 10^{-6}$. The latest value of Fafarman being $4{\cdot}6 \times 10^{-6}$/g. sec. sterad for the range interval (0–11)g./cm.^{2} of air equivalent.

In view of the above uncertainties and the difficulties of evaluating them, and also in view of the absence of any data for the meson flux at a low latitude it was considered worth while to find out this spectrum at Calcutta (12° N.) with a cloud chamber utilizing the ionization residual-range characteristic of slow muons. This method of finding out the muon flux involves only two corrections which can be simply evaluated. These corrections are (1) the estimation of the electron contribution to the muon spectrum when there is no absorber above the cloud chamber and (2) the estimation of the loss of particles due to the scattering out of the muons when absorbers of varying thicknesses are placed above the chamber. Both these estimations are based on theoretical formula involving the electro-magnetic interaction of the electron and the muon with the nuclear charge in the medium energy range where the validity of the equations have been established by various other experiments.

THE EXPERIMENT

The apparatus used in this experiment consisted of a cloud chamber which had a cylindrical illuminated region of 30 cm. diameter and 8 cm. deep. It was filled with argon to a pressure of 96 cm. Hg and illuminated by Xenon flash tubes placed behind pairs of condensing lenses. The chamber was fitted with five copper plates which stopped the muons. The top and bottom plates were $\frac{1}{4}$ inch thick and the three central ones $\frac{1}{2}$ inch. It was triggered by a threefold coincidence and anti-coincidence arrangement $C_1C_2C_3-A$ as shown in Fig. 1. The solid angle times area of the threefold counter arrangement was 0·677 cm.^{2}-steradian. This solid angle was completely covered by the anti-coincidence counters placed below the chamber. The efficiency of the threefold coincidence cum anti-coincidence circuit was tested with a pulse-generator and found to be 100%. The coincidence and anti-coincidence channels were of conventional design and the output pulses from them were independent of the pulse size from the G.M. counters. Stereoscopic photographs were taken and the ionization and scattering of particles were evaluated from both the views.

The experiment was divided into four parts. The first part contributed the range spectrum of the muons in the region 6·5–36·5 g./cm.^{2} air equivalent of absorber. The apparatus was then covered by a thin roof consisting of 0·075 cm. thick iron and 3·7 cm. Celotex. Other materials in the solid angle of the counter system were only the counter walls and counter trays. All these added together gave a total thickness of 6·5 g./cm.^{2} air equivalent which was the lowest range we could investigate. The upper limit of the range in this part of the experiment was determined by the total thickness of the first four copper plates inside the chamber. Particles stopping in the last plate were not counted because of inefficiency of the anti-coincidence system due to the emission of decay electrons, which could easily trip the anti-counters. Because of the small thickness of (6·5 g./cm.^{2} air equivalent) material above the chamber we assumed that the scattering effect of the particles during this part of the experiment was negligible.

The particles stopped in plate one were identified from their ionization characteristics only. In order to stop inside $\frac{1}{4}$ inch (5·5 g./cm.^{2}) copper plate a μ-meson should exhibit an ionization just greater than twice minimum while protons a much heavier ionization (> 5 times minimum). All particles stopping in plate one with minimum ionization were considered to be electrons. The particles stopped in other plates were identified

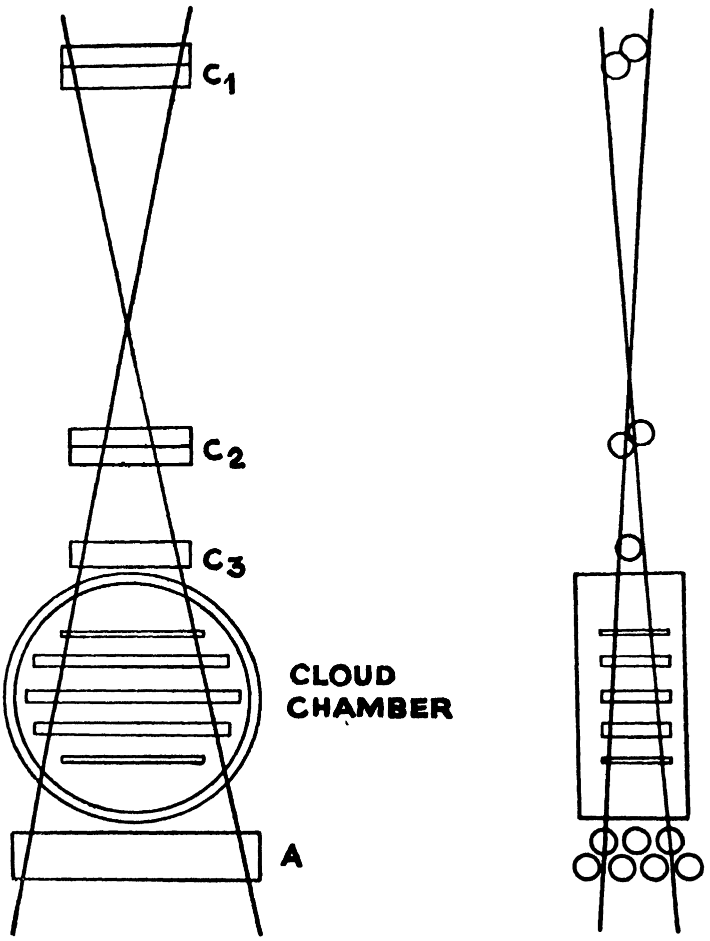

FIG. 1. Experimental arrangement of counters and cloud chamber for photographing stopped particle inside the chamber.

from both ionization in between and scattering angle at different plates. Any stopped particle which produced one or more secondaries in any plate was rejected as it was considered to be an electron. The contribution of non-showering electrons to the μ-meson spectrum was evaluated from the shower theory.

The second part of the experiment was performed to observe the higher energy region of the spectrum from 39·2–73·2 g./cm.^{2} air equivalent. A 5 cm. Pb absorber was now placed above the chamber, and the effect of scattering in this lead had to be taken into consideration. A considerable portion of muons while penetrating 5 cm. Pb would scatter out of the solid angle determined by the counter telescope and the cloud chamber and thus would not contribute to the measured meson flux. The ¼ inch top and bottom plates were now replaced by ½ inch thick copper plates in order to avoid a gap in the range interval when we moved from 5 to 10 and 15 cm. Pb absorber. All particles that entered the chamber accompanied by one or more particles were excluded, for they were considered to have originated either from soft electron showers or nuclear interactions inside *the 5 cm. Pb absorber.* As in the first part, the particles stopped in the last plate were excluded from analysis.

The third and fourth parts of the experiment were performed to obtain data for still higher energy regions of the spectrum by placing Pb absorbers of thicknesses 10 and 15 cm. successively above the chamber. The spectrum of muons within the region 72–106 g./cm.^{2} and 104–138 g./cm.^{2} air equivalent were studied from the pictures of stopped particles inside the chamber. The scattering corrections for these absorbers were made and the true spectrum determined.

RESULTS

During a total sensitive time of 850 hours 803 usable pictures were obtained. Of these 463 pictures showed single particles which stopped in one of the absorber plates inside the chamber. They were analysed further to obtain the μ-meson spectrum. The remaining pictures were mainly single electrons, small electron showers, low energy stars and particles of mass appreciably greater than that of a muon. A considerable part of the pictures showed particles scattered out by the chamber plates while at minimum ionization.

All particles which stopped in a plate showing distinct increase in ionization in the last gap were taken to be muons belonging to our range spectrum provided they have tripped the coincidence system. Particles which gave mass values 50% higher than that of a muon from residual range and ionization estimates were excluded. This might have caused a few pions to be included in the spectrum. But their number must be very small since pions produced in the absorber above would scarcely appear singly inside the chamber.

Fig. 2*a* shows the picture of a particle considerably heavier than a muon. Altogether ten such pictures were obtained during the whole experiment and critical analysis of their range and ionization is now in progress to determine their mass values accurately and the result of this analysis will be published separately. We give in Table I the distribution of these particles in time and range interval.

TABLE I

Distribution of particles of mass at least fifty per cent higher than that of a muon

Range interval g./cm.^{2} of air equiv.	Sensitive time in hours	Number of particles
6·5–36·26 ..	200	2
39·21–73·21 ..	210	5
71·96–105·95 ..	215	1
104·65–137·65 ..	225	2

Although the statistics is very poor, Table I shows a maximum of these particles in the range interval 39–73 g./cm.^{2} of air equivalent, i.e. with 5 cm. Pb above the chamber. These particles were excluded from the calculation of the muon spectrum. Fig. 2*b* shows a particle scattered out of the solid angle covered by the anti-counters by a plate inside the chamber and Fig. 2*c* a soft shower the secondaries of which are all stopped inside the chamber. All these pictures were of no use. Examples of useful pictures are shown in Figs. 2*d* and 2*e*. Fig. 2*d* shows two genuine stopped muons without decay electrons and Fig. 2*e* two μ-mesons with decay electrons.

After the counting of all stopped particles the data were corrected for (1) non-showering electron contribution when there was no lead absorber above the chamber and (2) the loss due to the scattering out of the particles when absorbers of varying thicknesses were placed above the chamber. The details of these calculations are given below.

The differential vertical range-spectrum was obtained from the expression given by York (1952).

$$I_v(R) = \left[\frac{1}{\sigma\Omega}\,\frac{1}{d}\right]\left[\frac{N(R)}{T}\right] \quad \cdots \quad \cdots \quad \cdots \quad \cdots \quad (1)$$

$I_v(R)$ represented the number of muons which arrive at sea-level per unit time per unit area within unit solid angle in the vertical direction and are brought to rest in one g./cm.^{2} of an absorber after traversing a thickness R of the same absorber.

$\sigma\Omega$ = solid angle area available to particles stopping in the chamber,
= 0·677 cm.^{2}-sterad in our case,
d = range interval in g./cm.^{2} of air equivalent,
$N(R)$ = number of particles stopped in the range interval,
and T = total time in seconds.

The total running time was corrected for the dead time of the cloud chamber cycle.

The range intervals were expressed in g./cm.^{2} of air equivalent so as to make it easier to compare with other results. The mass stopping powers of copper and lead relative to air were taken to be

Cu : 0·761
Pb : 0·574.

ESTIMATION OF CORRECTIONS

(i) *Correction for electron contribution*

The electron contribution to the muon flux was calculated in the following manner.

The minimum electron energies required for penetrating the various absorber plates (1, 2, 3, 4, etc.) inside the chamber were calculated by numerical integration of the curves for the rate of energy loss as a function of energy.

The total energy loss of an electron per cm. is given by the sum

$$-dE/dx = (-dE/dx)_{\text{rad}} + (-dE/dx)_{\text{coll}}.$$

$(-dE/dx)_{\text{rad}}$ was obtained from curves interpolated between those of Heitler (1944) for H_2O, Cu, and Pb. Fig. 3 shows the curve $(-dE/dx)$ in copper as a function of primary electron energy E-mc.^{2} in units of mc.^{2}.

The average range R of electrons with initial energy E_0 is given by

$$R = \int_1^{E_0} \frac{dE}{-dE/dx}.$$

The integration was carried out numerically from Fig. 3, and Fig. 4 shows the range of electrons in copper as function of its kinetic energy expressed in units of mc.^{2},

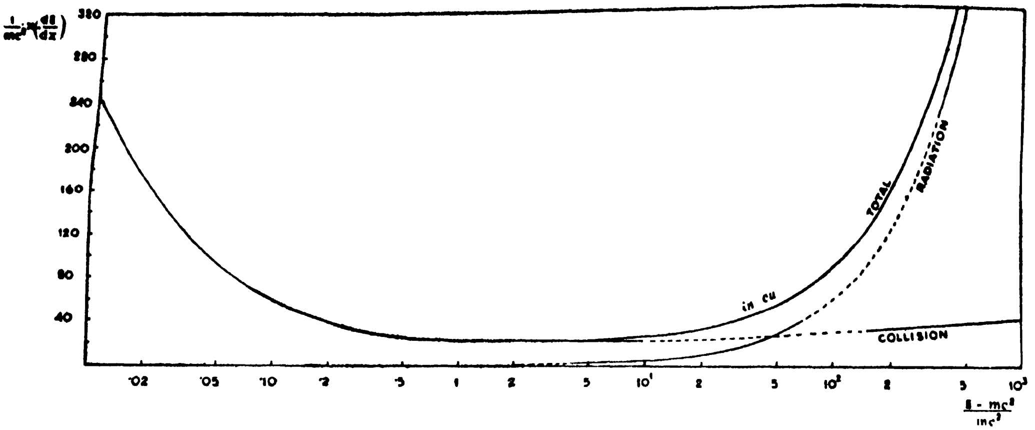

FIG. 3. Rate of energy loss of electrons in copper by radiation and by collision as a function of their kinetic energies expressed in units of mc.^{2}.

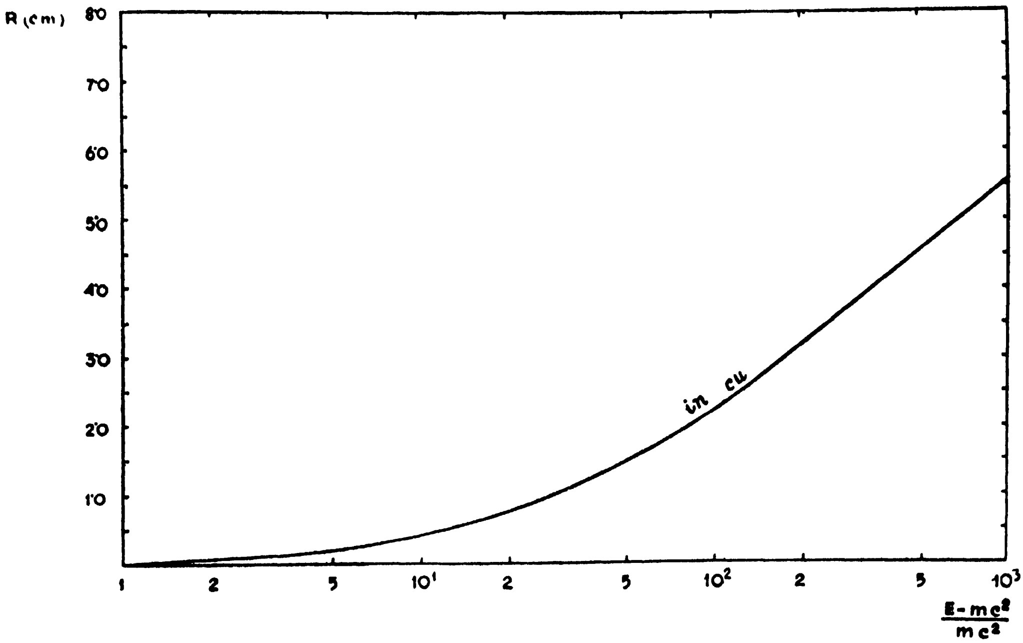

FIG. 4. Range of electrons in copper as function of their kinetic energies expressed in units of mc.^{2}.

Thus when there was no absorber above the chamber, the electrons which stopped at different plates inside the chamber would possess the following average initial energy when they entered the chamber.

Stopping in plate	*Initial energy*
2 ..	~20 Mev.
3 ..	~60 Mev.
4 ..	~140 Mev.

Thus we find that electrons which stop in Plates I and II have an energy less than the critical energy (21·8 Mev. for Cu) when they enter the chamber. No attempt has been made to find out the electron contribution in this energy region from the shower theory as the theoretical calculations in this region is uncertain by more than 20%. Particles stopping in Plate I, however, could be unambiguously distinguished from electrons by ionization alone. It will be seen in Fig. 6 that due to this uncertainty in the shower theory we have not been able to correct the muon spectrum in this range interval and there is a gap in the muon spectrum in the range interval 10·7 to 19·3 g./cm.^{2} of air equivalent.

In order to be mistaken for a meson stopped in the third plate an electron of energy 60 Mev. must not only stop in the third plate, but must also come out of the first two plates unaccompanied by secondary shower particle. The probability for this has been calculated assuming Poisson distribution. The probability that an electron of energy 60 Mev. penetrates the first plate (0·41 cascade units) without showering is 0·327. When it reaches the second plate (0·82 cascade units) it has an energy of only 37 Mev. and it has a negligible showering probability. But from Fig. 3 it is found that a 37 Mev. electron will have an average range of 18 g./cm.^{2} of Cu, and hence its probability of coming out of the second plate as a single particle is taken to be unity. Therefore the combined probability P of an electron stopping as a single particle in Plate III is 0·327. Exactly similar arguments lead to a value of P equal to 0·127 for particles stopping in Plate IV.

In order to determine how this probability would affect the muon spectrum we must know the relative intensity of muons to single electrons in this part of the spectrum. This will be evident from the following simple calculation. Let x and y be the number of electrons and muons in a certain range interval (energy intervals will be completely different), and let P be the probability that an electron stops in a certain plate unaccompanied by secondaries. Then $xP+y = N$, where N is the total number of single particles actually observed to stop in that plate. If we denote by r the ratio x/y, it is easily seen that

$$y = \frac{N}{rP+1}$$

gives the actual number of muons stopping in that plate. The calculation of r is carried out in two steps. First we find out from the data of Lombardo and Hazen (1945) the ratio of the number of electrons in a certain range interval to the total number of mesons of momentum greater than 130 Mev./c. Then we calculate the ratio of the number of mesons of the same range interval to the total meson spectrum from the data of Greisen (1943). The final results are given in Table II.

TABLE II

Percentage of mesons in the observed flux with no absorber above the chamber

Particle stopping in plate number	Range g./cm.^{2} air equiv.	(Number of electrons)/(Number of mesons)	Probability * for an electron to be mistaken for a meson	Percentage of mesons in the observed particle flux
				$(y/N)\times 100$
3	19·26–27·76	4·56	0·327	41·6
4	27·76–36·27	2·74	0·129	72·8

* We are indebted to Dr. S. K. Chakravarty for supplying us these values.

We have also determined from our experiment the ratio of the number of single particles stopping in Plate I with minimum ionization to those with more than twice minimum when the total absorber above the apparatus was 6·5 g./cm.^{2} of air equivalent. As the thickness of Plate I in this part of the experiment was 5·5 g./cm.^{2} of copper, this ratio gives an independent experimental value of r in the 6·5–10·7 g./cm.^{2} of air equivalent range interval. This value was 8·6 compared to the calculated value of 8 9 from Lombardo and Hazen and Greisen's curves. This agreement justifies to a certain extent our accepting the values of r from Lombardo and Hazen and Greisen's curves in the higher range intervals.

(ii) *Estimation of the scattered out particles*

It is well known that the coulomb scattering of singly charged particles varies inversely as their momentum and hence a large fraction of the muons in the low momentum range would surely be scattered out when lead absorbers are placed above the cloud chamber. The scattering effect would not only depend on the thickness of the absorber but also on its distance from the spot where the scattered particles are required to be incident in spite of passing through the absorber. In our case the particles have to pass through the absorber, the G.M. counter number 3 (Fig. 1) and then stop in one of the plates inside the chamber. Hence, assuming they are muons, we know the momentum of the incident particles when they enter the absorber and we take the momentum of the particle in the last gap to be equivalent to a range equal to half the thickness of the plate in which it stops. Under these circumstances, the mean square projected angle of scattering considering energy losses in the absorber has been given by Rossi and Greisen (1941) and is given by

$$\alpha^2 = \left\langle \theta^2 \right\rangle_{AV(t)} = \tfrac{1}{2}\left\langle \Theta^2 \right\rangle_{AV(t)} = \tfrac{1}{2}\left\{\frac{E_s^2}{\mu^2}.t.\frac{\log\left[\frac{1+y_2}{1+y_1}\right]_{p_2}}{\left(y_1+\frac{1}{y_1}\right)-\left(y_2+\frac{1}{y_2}\right)}\right\} \quad .. \quad (2)$$

where α^2 = mean square of the projected angles of scattering θ in radians,

Θ^2 = square of the actual angle of scattering,

E_s = 21 Mev.,

μ = 107 Mev. for the muon mass,

y = $[(p/\mu)+1]^{\frac{1}{2}}$,

and t = thickness of the scatterer in radiation lengths and p_1 and p_2 are momenta of the particles at the time of entering and leaving the absorber.

When lead absorbers of varying thicknesses were placed above the chamber the plates inside the chamber were 11·0 g./cm.^{2} thick and hence p_1 always correspond to a residual range equal to the total thickness of the lead absorber and the copper plates penetrated plus 5·5 g./cm.^{2} of copper. The total thickness is then increased by 5% in order to compensate for the zigzag nature of the particles inside the absorber and their inclination to the vertical. The values of $2\alpha^2$ for different thicknesses of lead absorber and for particles stopping in different plates are shown in Table III. Because of this scattering the particles, which would have otherwise reached the particular plate of the cloud chamber, would no longer be going in the right direction so as to hit the plate within the illuminated volume. As a result the particle will be lost to the apparatus. In order to calculate the percentage loss (or rather the percentage received) of the particles, it

TABLE III

Distribution of the mean square projected angle of scattering for various particles stopping inside the chamber

For particles stopping in plate number	Total thickness of the scatterer in rad. lengths	$2\left\langle \theta^2 \right\rangle_t \quad \left\langle \Theta^2 \right\rangle_{AV(t)}$ radians
1	10·07	0·470
2	10·89	0·451
3	11·71	0·416
4	12·53	0·387
1	19·73	0·556
2	20·55	0·528
3	21·37	0·502
4	22·19	0·476
1	29·39	0·601
2	30·21	0·586
3	31·03	0·544
4	31·85	0·543

has been assumed that all the particles after passing through the absorber above the chamber would hit the counter C_3 plated just below it. Actually, however, there will be some particles which would, as a result of scattering, never reach counter C_3 although they had crossed C_1 and C_2. But this effect will be compensated by those which had crossed counter C_3 only as a result of scattering in the absorber and would not have done so had there been no scattering. This latter effect has been shown by Germain (1950) to be less than 0·36% for 15 cm. of Pb placed above the chamber. Now the distribution of the scattering angle Θ and therefore the projected angle of scattering follows the normal Gaussian distribution with the root mean square angle α as given by equation (2). Hence the probability that a certain value of the projected angle lies between θ and $\theta + d\theta$ is given by

$$P = \frac{1}{\sqrt{2\pi}} \cdot \frac{1}{\alpha} \cdot e^{-\theta^2/2\alpha^2} \, d\theta$$

$$= \frac{1}{\sqrt{\pi}} \cdot e^{-\theta^2/2\alpha^2} \, d(\theta/\sqrt{2}\alpha) \qquad \ldots \quad \ldots \quad \ldots \quad (3)$$

In order to calculate the fraction of particles received at a particular plate, we have to integrate the above expression between the limits $\pm\theta_1/\sqrt{2}\alpha$, where θ_1 is given by the geometry of the counter placed just below the lead absorber and the particular plate in which the particles have stopped inside the chamber. It will be noticed that the absorber was placed between C_2 and C_3 when its thickness was only 5 cm. and due to lack of space it was shifted to the space between C_1 and C_2 when it was 10 and 15 cm. thick. Hence the value of θ_1 is obtained by joining the extremities of the effective length of either C_2 or C_3 placed just below the lead absorber, and the ends of the plate concerned, as shown in Fig. 5. Here we assume that all particles incident inside the solid angle covered by the threefold coincidence unit, reach the counter placed just below even after penetrating the absorber.

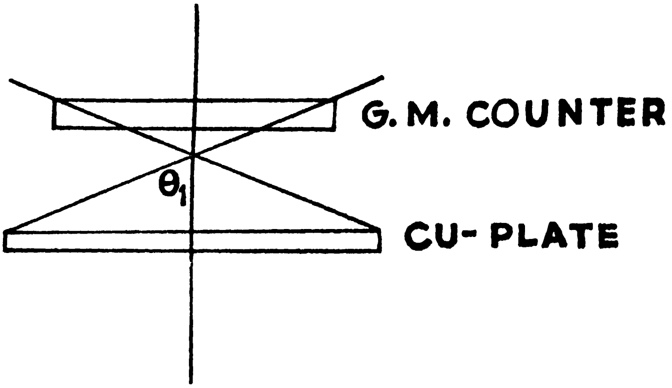

FIG. 5. Arrangement showing the angle subtended by the counter placed just below the absorber with the relevant cu-plate.

Thus we calculate the expression

$$\frac{1}{\sqrt{\pi}}\int_{-x_1}^{+x_1} e^{-x^2}dx = \frac{2}{\sqrt{r}}\int_{0}^{x_1} e^{-x^2}dx, \quad \ldots \quad \ldots \quad \ldots \quad (4)$$

where $x_1 = \theta_1/\sqrt{2}\alpha$
for every plate inside the chamber and thus obtain the fraction of particles of such energy that they should stop at that plate and would go at such an angle that they get to the plate, i.e. their projected angles of scattering lie between $\pm\theta_1$ (Fig. 5). The evaluated fraction of particles received for different absorber thickness and for the different plates inside the chamber are given in Table IV.

TABLE IV

Evaluated fraction of particles received under different thickness of absorber

Absorber thickness above the chamber cm. Pb	Particles stopping in plate	θ_1 radian	$\theta_1/2 = x_1$	Fraction of particles received $\frac{2}{\sqrt{\pi}}\int_0^{x^1} e^{-x^2}\,dx$
5	1	0·942	1·37	0·947
5	2	0·907	1·35	0·944
5	3	0·907	1·40	0·952
5	4	0·768	1·23	0·918
10	1	0·558	0·75	0·710
10	2	0·558	0·77	0·721
10	3	0·558	0·79	0·735
10	4	0·471	0·68	0·664
15	1	0·558	0·72	0·690
15	2	0·558	0·73	0·697
15	3	0·558	0·76	0·716
15	4	0·471	0·64	0·634

The 10 and 15 cm. Pb absorbers were placed between counters C_1 and C_2 (Fig. 1) whereas the 5 cm. Pb absorber was placed below C_3 and this results in a sharp fall of the fraction of muons received under 10 and 15 cm. of Pb, because the angle θ_1 decreases when the absorber is moved up.

DISCUSSION OF RESULTS

The observed number of particles were corrected for (1) electron contribution, (2) presence of heavy particles, and (3) loss of particles due to scattering by the methods explained above and the final corrected intensity I_v is given in the last column of Table V.

TABLE V

Absolute differential range spectrum of muons at 12° N. after corrections

Range interval g./cm.^{2} air equiv. R	Observed number of stopped particles	Corrected number of muons N(R)	Total sensitive time in hours	Vertical differential muon intensity g^{-1} sec^{-1} $ster^{-1} \times 10^6$
6·5– 10·8	$61(I/I_m = 1) +$ $8(I/I_m \geq 2)$	8	200	8·85 ± 0·91
19·3– 27·8	53	21·41	200	5·17 ± 0·75
27·8– 36·3	32	21·64	200	5·22 ± 0·74
39·2– 47·7	23	24·26	210	5·59 ± 0·75
47·7– 56·2	22	23·30	210	5·36 ± 0·74
56·2– 64·7	28	28·35	210	6·52 ± 0·81
64·7– 73·2	26	23·96	210	5·50 ± 0·74
72·0– 80·5	20	28·17	215	6·32 ± 0·77
80·5– 89·0	19	26·35	215	5·92 ± 0·77
89·0– 97·5	18	24·49	215	5·50 ± 0·73
97·5–106·0	21	30·11	215	6·74 ± 0·83
105·9–113·2	18	26·08	225	5·60 ± 0·73
113·2–121·7	16	22·96	225	4·93 ± 0·69
121·7–129·2	21	26·54	225	5·70 ± 0·74
129·2–137·7	20	31·55	225	6·77 ± 0·80

The data are plotted in Fig. 6 as a histogram in which the horizontal side of each rectangle represents the range interval and the vertical side the probable statistical error $(0{\cdot}67\sqrt{n})$

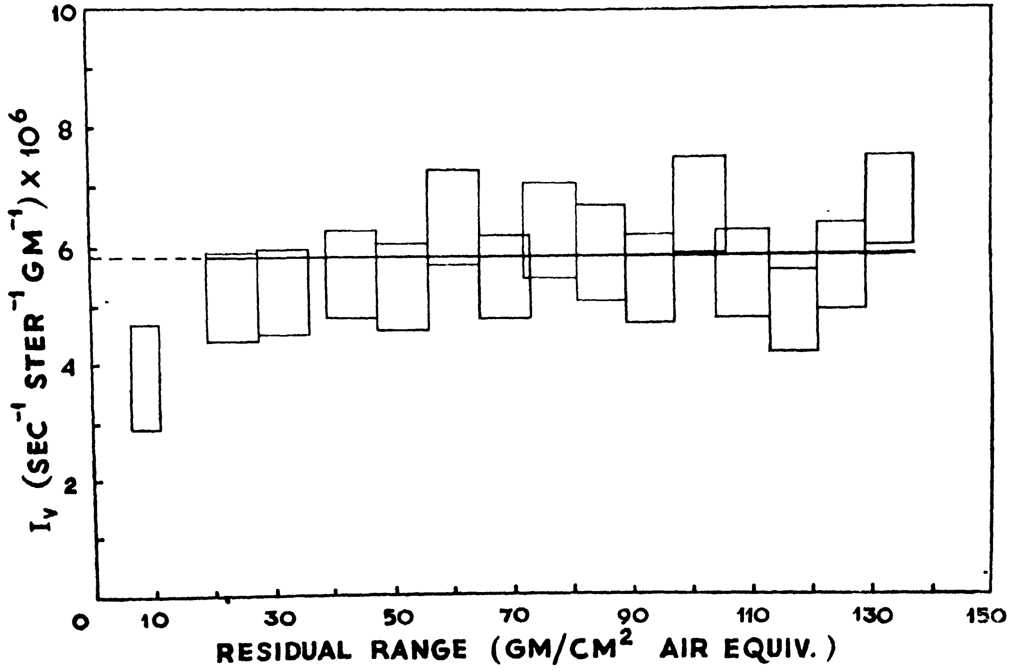

FIG. 6. Histogram of the flux of muons with residual range at 12° N. (geomagnetic latitude).

in the intensity in that interval. It will be seen that the range-spectrum shows almost a constant intensity of muons from 20-140 g./cm.^{2} of air equivalent at 12° N., indicating a flat spectrum in this interval. The considerable decrease of the intensity in the lowest interval (6·5–10·7 g./cm.^{2}), although not outside statistical deviation, seems to be real. The gap in the spectrum in the interval 10·7–19·0 g./cm.^{2} is due to our inability to find out the electron contribution in this portion of the spectrum from the shower theory and the small gap between 36 and 39 g. is due to non-overlapping of the absorber thickness when 5 cm. of Pb was placed above the chamber. This latter gap has been avoided for 10 and 15 cm. Pb absorbers by increasing subsequently the total plate thickness inside the cloud chamber.

If we accept that the muon spectrum between 20–140 g./cm.^{2} is flat, we can evaluate the mean intensity in this interval with a much better statistical accuracy. This value comes out to be 5·78 ± 0·20 g^{-1} cm.$^{-1}$ ster^{-1}. In comparing .our results with those previously obtained, we notice that all measurements of the muon intensity in the low energy region are confined to latitudes 42°–50° N. with the exception of Del Rosario and Davila-Aponte (1952) who have measured this spectrum at 29° N. But the absolute values obtained by these authors have not been corrected for the scattering effect. Their average intensity of 4·18 ± 0·20 for the interval 10-140 g./cm.^{2} would be increased by about 40% if the scattering effect of the lead above is taken into consideration. This leads to a value of 5·86 ± 0·26 for the spectrum at 29° N.

The determination of the muon flux by the cloud chamber method by Lichtenstein at 45° N., has also not been corrected for the scattering effect by 8·8 inches of carbon placed at a fairly large distance from the range chamber. The loss of particles due to scattering had been further augmented in Lichtenstein's experiment due to the presence of a magnetic field above the range chamber. The magnetic field removes from the solid angle of the range chamber particles of low momentum which appear in the momentum chamber inclined to the vertical due to the scattering in the carbon plate. Even if we exclude the effect of this magnetic field we find that 12% to 18% of muons have been lost in Lichtenstein's experiment in the range interval 20–140 g./cm.^{2}. This correction raises Lichtenstein's average value of 6·2 ± 0·2 to 7·1 ± 0·3 at 45° N. The best measurement of the muon flux is, in our opinion, due to D. Brini *et al.* (1955), who have made a rigorous and detailed calculation of the scattering effect and find a small slope

TABLE VI

Absolute values of the muon intensity at different latitudes

Author	Geomagnetic latitude	Mean intensity I_v	Range interval in g./cm.^{2} of air equivalent
1. Lichtenstein (after scattering correction) ..	45° N.	7·12 ± 0·26	20–140
2. D. Brini *et al.* ..	42° N.	6·78 ± 0·6	20–140
3. Rosario and Davila-Aponte (after scattering correction) ..	29° N.	5·86 ± 0·26	20–140
4. Basu and Sinha ..	12° N.	5·78 ± 0·20	20–140

0·091 in the μ-meson spectrum at 42° N. (Bologna). If we disregard this small slope and evaluate the mean intensity for the spectrum in the range interval 20–140 g./cm.^{2}, we get the value 6·78 ± 0·6 from Brini *et al.*'s data. We collect these results in Table VI. We see from Table VI, that the vertical muon flux exhibits a latitude effect of about 15 ± 4% from 45° to 12° N. and if plotted the data shows a knee similar to the total intensity of the hard component. More data at other latitudes are needed to establish this effect with certainty. The experiment is in progress for collecting data with improved statistics and at larger range intervals.

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ABSTRACT

The differential vertical intensity of μ-mesons at sea-level has been investigated at 12° N. geomagnetic latitude (Calcutta) in the range interval 6–140 g./cm.^{2} of air equivalent. A multi-plate cloud chamber triggered by a threefold coincidence and anti-coincidence counter system was used for this purpose. In the range interval studied the spectrum obtained after various corrections has been found to be nearly flat, the mean intensity being 5·78 ± 0·20/·10^{-6}/g. sec. sterad. Comparison with results obtained by others at higher latitudes indicates a fall in this intensity by 10–15% from 45° N. to 12° N.

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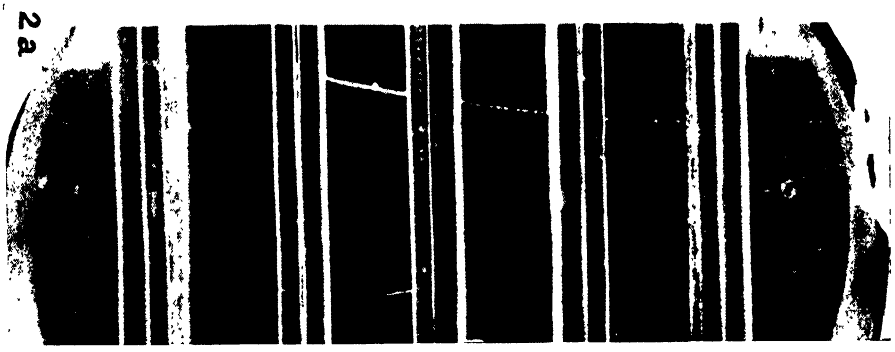
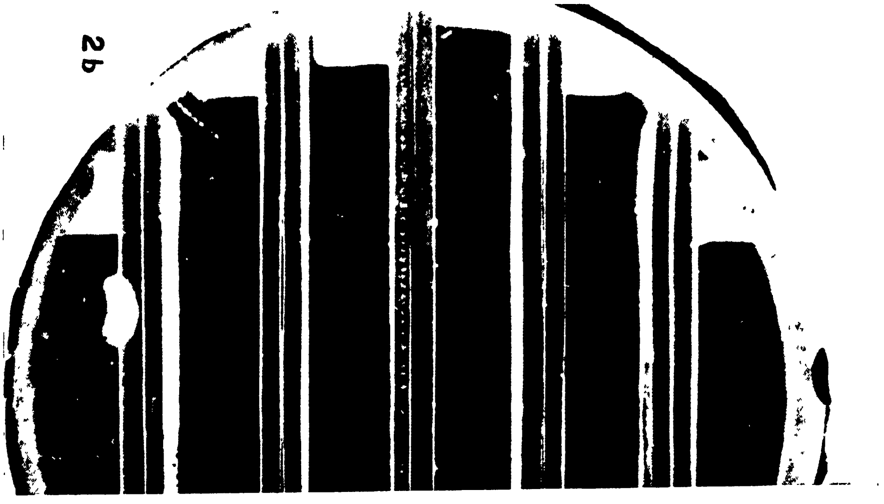




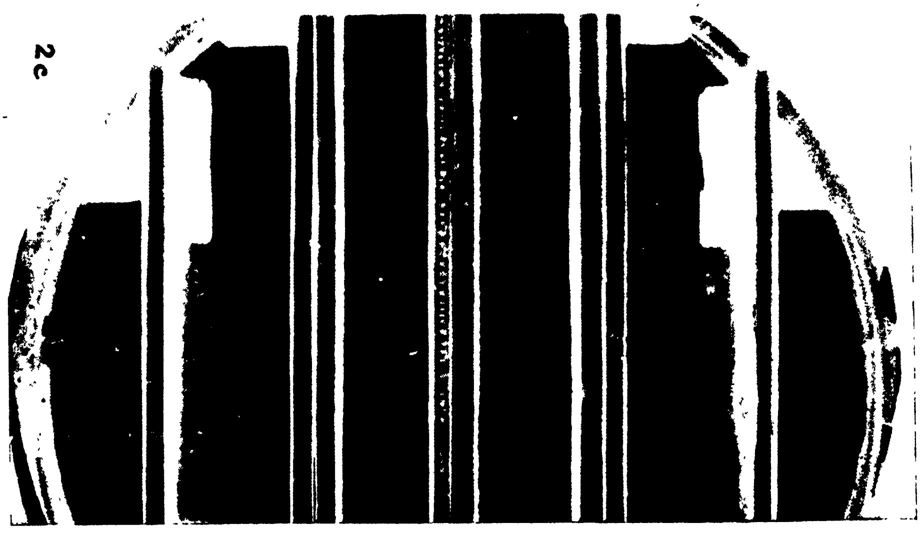
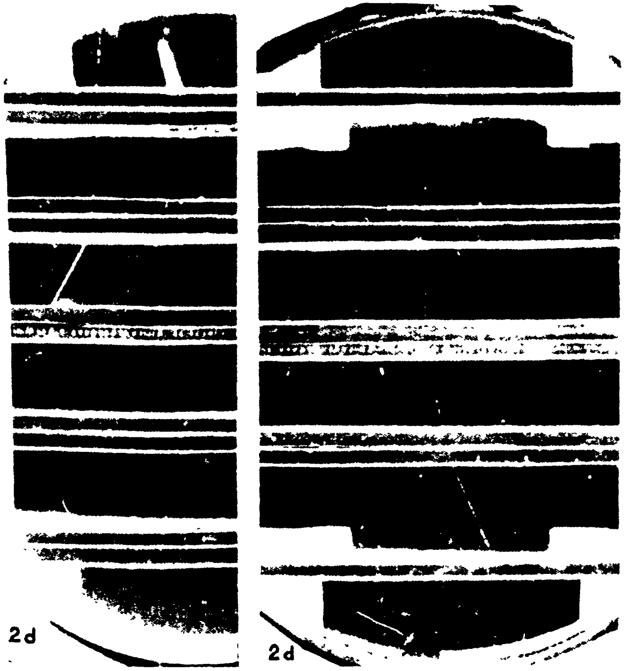


FIG. 2*a*. Track of a stopped particle considerably heavier than a μ-meson.
FIG. 2*b*. Track of a particle scattered out from the solid angle covered by the anti-counters.
FIG. 2*c*. A low energy shower all the particles of which have stopped.



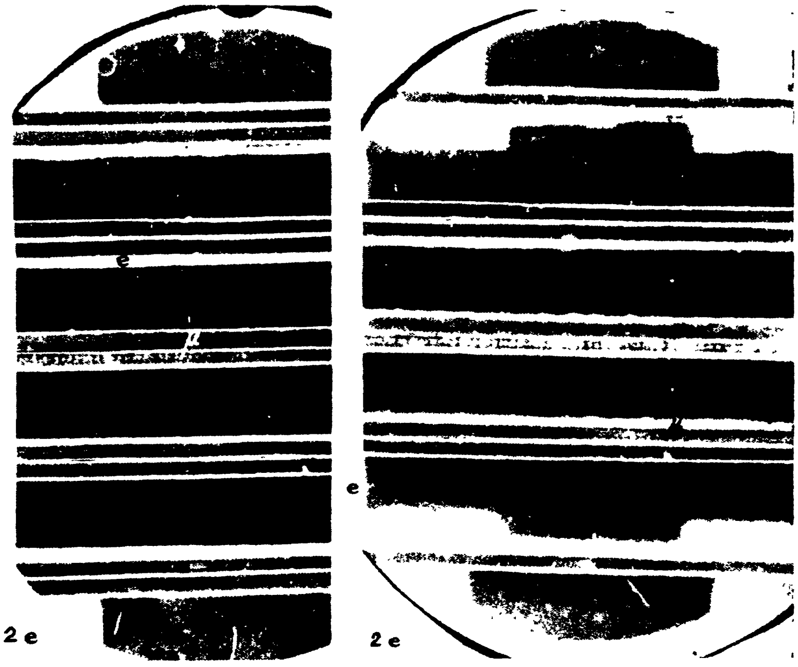


Fig. 2d. Tracks of two genuine μ-mesons stopped inside the chamber without decay electrons.

Fig. 2e. Tracks of two stopped μ-mesons with decay electrons

X. PART I. ON THE MEASUREMENT OF ULTRASONIC POWER RADIATED FROM A QUARTZ CRYSTAL TRANSDUCER INTO LIQUID MEDIUM * †

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ABSTRACT

This paper describes an experimental method of determining the acoustic radiation force and the streaming force separately. By extrapolation, acoustic intensity at the source is determined.

Unlike Borgnis and Cady and Gittings, the sum of the acoustic and streaming forces, instead of being independent of distance from the source, is found to decrease at a slow rate up to 8 cm. and henceforth exponentially.

As absorber, a cavity radiometer was used. This acted as a perfect absorber.

The reacting force on the transducer was also measured and found, within the limits of experiment, to be equal to that on the absorber. This promises an independent method of measuring acoustic power without using any absorber.

From the exponential decrease curve of the radiation force, α the attenuation constant of the experimental liquid is determined.

1. INTRODUCTION

In previous papers, the author (1953, 1955) has described in detail the methods of measuring the output acoustic power of a crystal transducer by (i) calorimetric substitution method, and (ii) spherical radiometer method, in which primary attention was focused on the determination of acoustic energy density in liquid medium. To assess the merit of the calorimetric substitution method for determining the acoustic power radiated by a quartz crystal transducer, it is necessary to discover how far the acoustic energy dissipated in liquids converts directly into heat. Although measurements have been made of temperature rise produced in liquid during irradiation by ultrasonics, yet there remains much to be investigated in detail in this line to reach a definite conclusion. Due to the passage of ultrasonic through any liquid, there are admittedly complications in such experiments because of variations in dielectric constant, thermal conductivity and specific impedance but these variations are not great. The measure of acoustic power radiated as obtained by calorimetric substitution method is only 35 per cent (approx.) of the input electrical power to the crystal transducer, and no satisfactory explanation of the rest 65 per cent has yet emerged. So, in the next endeavour, acoustic power radiated in liquid was measured by means of spherical radiometer method. The theory underlying this method has been developed by King (1934). The acoustic energy density as measured by this method was found to be about 10–14 per cent larger than that as obtained by the calorimetric substitution method.

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* Part of the results reported to Ann. Rept. Bose Institute, 1954-55.

† This paper contains the essential results of a thesis approved by the Calcutta University for the degree of Doctor of Philosophy (1957).

The radiation pressure method, in any form whatsoever, also is not without complications. The measurements of acoustic output power by radiation pressure method involves considerable complications. These are caused by the fact that acoustic field sets up a steady streaming in the medium. Two effects are supposed to be responsible for the mass flow: (1) 'pumping effect' which may occur at the source and by which fluid is sucked into the beam and set in motion in the direction of the wave propagation; (2) owing to viscous forces, the wave generates what is called 'dynamic flow' and a steady vortical motion is set up in the fluid causing steady streaming along the beam in the direction of propagation of the incident wave and returning outside the beam. The phenomenon of dynamic flow is inseparably associated with acoustic radiation in a fluid. Streaming is an effect of second order. The problem of streaming has been thoroughly treated taking into account at least second order terms in the hydrodynamic equations by C. Eckart (1948), J. J. Markham (1952), P. J. Westervelt (1953), and W. L. Nyborg (1953).

So, while any attempt is made to measure acoustic energy density by radiation pressure method, these forces—acoustic and streaming—must be separated one from the other and must be measured independently so as to get some clue to the unaccounted dissipation input electrical power to the crystal transducer. Common practice of separating out the acoustic and the streaming forces is to use a thin membrane, usually cellophane or collodion sheet, which is transparent to radiation but opaque to streaming. This membrane is placed parallel to the transducer face but very close to the radiation detecting element.

Next, the choosing of the acoustic energy detecting element has long plagued the investigators in this line. When a disc is placed in the sonic field to measure acoustic intensity, the field pattern gets changed and various complications arise. So, in the ideal practical condition, the disc must behave either as a perfect absorber or a reflector of acoustic wave and under this condition, energy density can be calculated in a simplified way. But practically, it is very difficult to achieve these qualities in a disc. That is the reason why the plate or radiometer for use in liquids is sometimes made of light mica disc with a thin layer of air in between, for almost perfect reflection is obtained from liquid to air. By utilizing a device like this, acoustic energy density alone may be measured accurately but the streaming energy density cannot be measured.

A new and illuminating approach to this effect was made by L. N. Liebermann (1949) to separate out the acoustic and the streaming forces and to determine the magnitudes. He differentiated these two forces by dint of his well-known experimental arrangement which makes use of the inertia of streaming. While radiation force responds instantly to abrupt changes in sound intensity, the streaming velocity has a relatively long time constant. But his experiment has been criticized by some workers based on the experiments of Darner and Lide (1954) that streaming is very rapidly established and that a difference in the time of development of the full effects cannot be used to distinguish between radiation pressure and streaming, acting on the vane which registers the forward pressure. Even in spite of that, his experiments have got a great bearing in focusing the attention of the future workers to the possibilities of this type of measurements. The objection that has been raised against this method may be valid only when the distance between the source of ultrasonic energy and the detecting vane is very small. As the distance increases, the difference in the time of development of the full effects also increases on account of the fact that ultrasonic wave travels, say in water, with a velocity of about $1{\cdot}5\times10^5$ cm./s, whereas the velocity of streaming is about 3 to 4 cm. per sec. and so there should be an appreciable time lag in the development of the full effects. This time lag effect has also been observed in the present investigation in which a different measuring apparatus was used. Even Darner and Lide reported a time lag of the order of 1/50 s. Of course, this time delay of 1/50 s. is subject to the experimental accuracy of synchronization of the switching of the oscillator and the opening of the shutter of the camera and also upon the time of the exposure to take the photograph of the effect produced due to streaming. Discussions of these points are not made in the paper of Darner and Lide. So it is too early a stage to conclude about the simultaneous development of radiation and streaming pressures. They, in their paper, reported that measurements at the U.S. Navy under-water sound reference laboratory had shown the sonic wind to develop quite definitely in 1/50 s. or less.

Besides these, there exists an 'after effect' in the liquid medium. Of course, the duration time of the 'after effect' varies with different liquids.

W. G. Cady and C. E. Gittings (1953) designed an apparatus with improved technique and sounder theoretical basis to measure the acoustic and streaming forces separately with sufficient accuracy with a view to verify the postulate of F. E. Borgnis (1953) that, under certain experimental conditions, the sum of the forces—acoustic and streaming—on absorber of a beam of plane waves in an attenuating liquid is independent of distance from the source. They verified this postulate by dint of their experiment at least over the range 0–25 cm. (approx.).

In the present investigation, an apparatus, similar to that used by Cady and Gittings, was employed to measure the radiation and the streaming forces separately. Unlike Cady and Gittings, in the present case in water medium, the radiation force was found to decrease exponentially with distance, whereas the streaming force, after increasing exponentially up to a distance of about 8 cm., decreases exponentially. The sum of these forces as obtained by adding up the individual magnitudes was found, after decreasing very slowly in a linear way up to a distance of about 8 cm., to decrease exponentially and this is the result to be expected generally on account of the production of vorticity in the liquid.

This apparatus with some minor changes was used to measure the acoustic power radiated from a single measurement, based on the Newton's law, that action and reaction are equal and opposite.

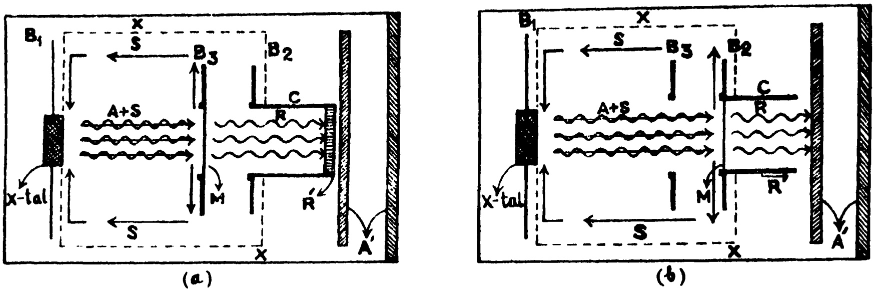
From the measurements of radiation forces, the attenuation coefficient α of acoustic energy in liquid was calculated.

Experiments were performed at a frequency of 11 Mc/s. (harmonic mode of vibration of crystal) and the liquid investigated was water.

2. THEORETICAL CONSIDERATIONS OF ACOUSTIC ENERGY DENSITY ANYWHERE IN THE PATH OF THE ACOUSTIC BEAM

The physical approach to the propagation mechanism is shown in Fig. 1.

An attempt has been made to represent in a very rough way the acoustic radiation and the streaming propagation mechanism and their trajectories in the figure. Measurements are based on the idea that gradients in streaming velocity can give rise to pressure which, as well as the pressure of radiation, can exert a force on an object placed in the path of rays. Liebermann did apply this principle to distinguish these two causes by measuring the force on a vane in the path of streaming just after radiation was turned on. In the present investigation, time average forces acting on the vane were measured by the



(a) (b)

Fig. 1, FIGURES (a) AND (b) SHOW THE SCHEMATIC REPRESENTATION OF THE PHYSICAL PROCESS UNDERLYING THE MEASUREMENTS OF RADIATION AND STREAMING PRESSURES RESPECTIVELY.

B_1, B_2 and B_3—baffles to hold the transducer *X-tal*, membrane *M* and cavity *C*. *A'* and *R'*—acoustic energy absorbers; *A* and *S*—acoustic radiation and streaming; *S*—streaming; *R*—radiation; *X*—shows undisturbed region. (Absorber *A'* just behind the cavity is movable and that at the end of the tank is fixed.)

application of this principle. Following conditions are to be satisfied in carrying out the measurements effectively.

(1) There must be a device to separate acoustic radiation from streaming without much diminution of either, while measuring.

(2) Radiation detecting element must act as a practically feasible ideal absorber.

(3) Streaming after striking the vane must move after being diverted at right angles to the incident direction.

(4) The dimensions of the tank must be made sufficiently large in comparison with the cross-section of the beam so as to leave sufficient undisturbed region for the return flow of liquid.

There must not be any streaming behind the baffle containing the absorber, but this is likely to be present, as the acoustic radiation comes out of the absorbing tube while measuring the force due to streaming. This is to be reduced to minimum by placing an absorber (Figs. 1*a* and 1*b*) just near the open end of the absorbing tube.

The above conditions being fulfilled, the total time average pressure on the absorber may be expressed in the following way:

$$<P> = p_o(x) + <p_a(x)> + <p_s(x)> \quad .. \quad .. \quad .. \quad (1)^*$$

where $<\ >$, the angular parentheses, denote the time average value,

p_o—hydrostatic pressure at x,

$< p_a >$—time average acoustic radiation pressure at x,

$< p_s >$—time average streaming pressure at x.

In the absence of acoustic radiation, the total pressure $< P >$ reduces to p_o—that is, the liquid is under the hydrostatic pressure only. Further, here the term p_o includes, besides hydrostatic pressure, other pressures of small magnitudes arising out of other causes. From the relation (1), it is clear that the total excess pressure $<p_a> + <p_s>$ and $< p_a >$ and $< p_s >$ can be measured independently simply by choosing the experimental conditions.

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* $< p_a(x) >$ stands for the expression $\int \rho_a U_a dA$, and $< p_s(x) >$ for $\int \rho_s U_s dA$, where ρ_a, ρ_s are the variations of densities due to radiation and streaming respectively; U_a, U_s—radiation and streaming velocities respectively.

3. APPARATUS AND EXPERIMENTAL ARRANGEMENT

(*a*) *Measuring Device*

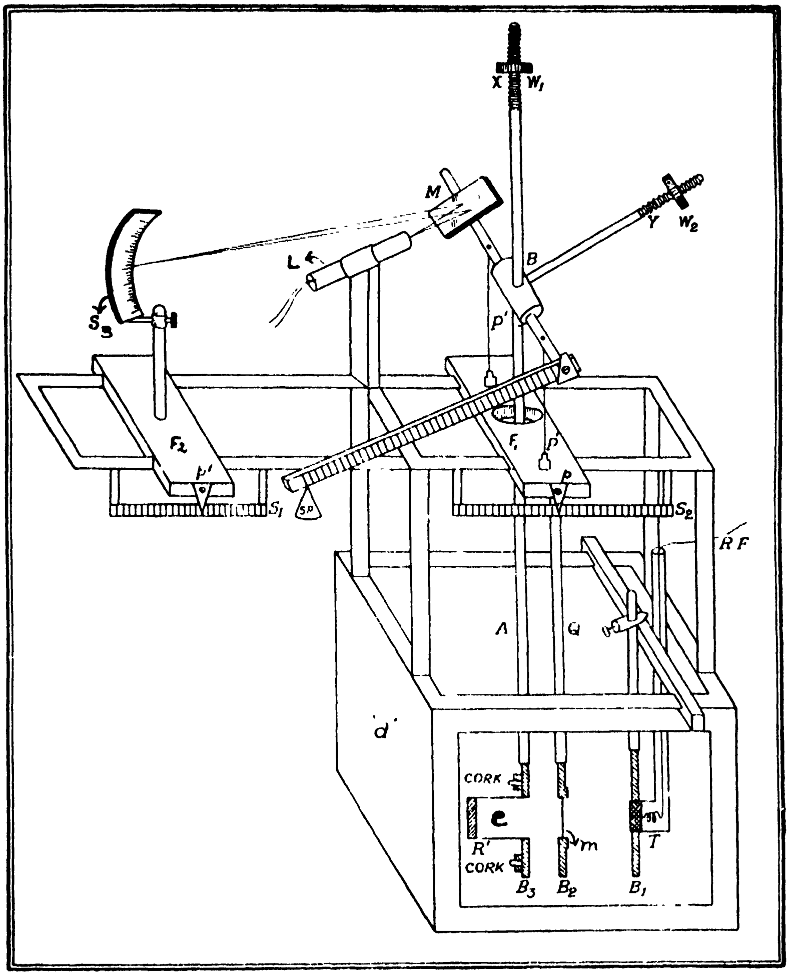

FIG. 2. Figure shows the schematic experimental arrangements for the mechanical and optical systems. '*a*'—experiment tank; B_1, B_2 and B_3—baffles to hold the transducer *T*, membrane *m* and cavity *C* respectively; *A*—a rod connecting the baffle B_3 to the cross-bar *B*; *Q*—a rod fixing the baffle B_2 to the movable platform F_1; *R'*—acoustic energy absorbing material; S_1, S_2 and S_3—scales; *p* and *p'*—pointers attached to the platforms F_1 and F_2; *M*—mirror; *L*—lamp; *P'*—hard steel pivots; *R.F.*—connection for input r.f. voltage; *X* and *Y*—threaded rods for adjusting sensitivity and balance by weights W_1 and W_2; *Sp*—scale-pan.

The transducer *T* (Fig. 2) with its baffle B_1 of brass 15×20 cm.^{2} was mounted at one end of a tank containing distilled water. A screw system arrangement was made to move the baffle B_1, with the transducer, in lateral directions as well as in the upward and downward directions and provision was made for rotating the plane of the transducer about the horizontally mounted cross-bar. The distance between the source and the cavity was varied in the range 3 cm. to 18 cm. The cavity tube used as an absorber, with the rubber stopper *R'*, was held by a rod *A* which extended into the water from above. In the present investigations, B_2 and B_3 measured 11×11 cm.^{2}. Both were immersed into water from above. In order to get rid of reflections from the remote end of the tank, thick rubber sheet and cork sheet were stretched on the end wall.

A cross-bar, to the centre of which a rod A is attached for holding the absorbing or the transmitting system, is supported at each end by hardened steel pivot P', resting in a jewel groove. X and Y are two threaded rods positioned at the centre of B, X being in continuation of A projecting upwards. Y is at right angles to A. Counter-weights W_1 and W_2 on the threaded rods serve the purpose of adjusting the sensitivity and the balance. A mirror M is attached to measure the amount of rotation produced. At the front end of the cross-bar a graduated scale is attached. By adjusting the position of the rider R (not shown in Fig. 2)—of known mass—on the scale, a balance in the system can be achieved. In the present investigation, instead of the rider a scale-pan was attached at the end of the scale. Balance was made by adding small weights on the scale-pan.

The jewel grooves are set on a platform having a central hole for allowing the rod A to pass through. The diameter of the hole is such that the rod A does not touch the platform at the time of maximum deflection. The platform as a whole moves on two rails. A pointer p, fixed in the platform, glides along a scale S_2 to show the position of the absorber from the source of ultrasonic energy.

The light spot reflected by the mirror M is received on a scale S_3 placed at a distance of 1 metre from the mirror. The scale-stand also moves on two rails in response to the movement of the platform as described above.

The total weight of the suspended system is 150 gm. Corks are attached to the baffle attached to A, so as to minimize the pressure on the jewel grooves and thereby increasing the sensitivity of the system.

Another rod Q, similar to A, is clamped in the movable platform to hold the third baffle B_3, the dimensions of which are the same as those of the second baffle, i.e. the baffle to which the cavity C is attached. This rod is fixed at a distance of 1 cm. from the rod A. This third baffle is for holding the screen.

When the crystal oscillated, the force on C (Fig. 2) caused the suspended system to rotate about the pivots. A known restoring torque was then applied to bring C back to its original position as indicated by the mirror M (Fig. 2) and an optical lever. The torque was applied by adding known weights on the scale-pan. From the mass added on the scale-pan, the force on the vane and hence the ultrasonic energy density was determined.

The membrane m was made of a film of collodion. A dilute solution of collodion in ether was spread over a glass plate. It was allowed to dry. The glass plate was then immersed in water for some time and the film was carefully pried off under water. This film was fixed to the baffles by means of ring washers and screws. The film was so thin that practically no reduction in the force was detected. A thin sheet of cellophane is also used in some experiments.

While measuring the acoustic intensity from a single measurement by utilizing the Newton's law, 'Action and reaction are always equal and opposite', the jewel grooves were replaced by two highly polished steel grooves so as to get the continuity in the path of the electrical current flowing through the transducer. The crystal transducer was attached to the central rod A, in place of the absorbing system. R.F. power was fed to the transducer by means of a very flexible wire in such a way as to make the effect of the frictional resistance due to this wire connection in the free-movement of transducer system negligible.

Actual experimental arrangements are shown by means of the photograph Fig. 2*a*.

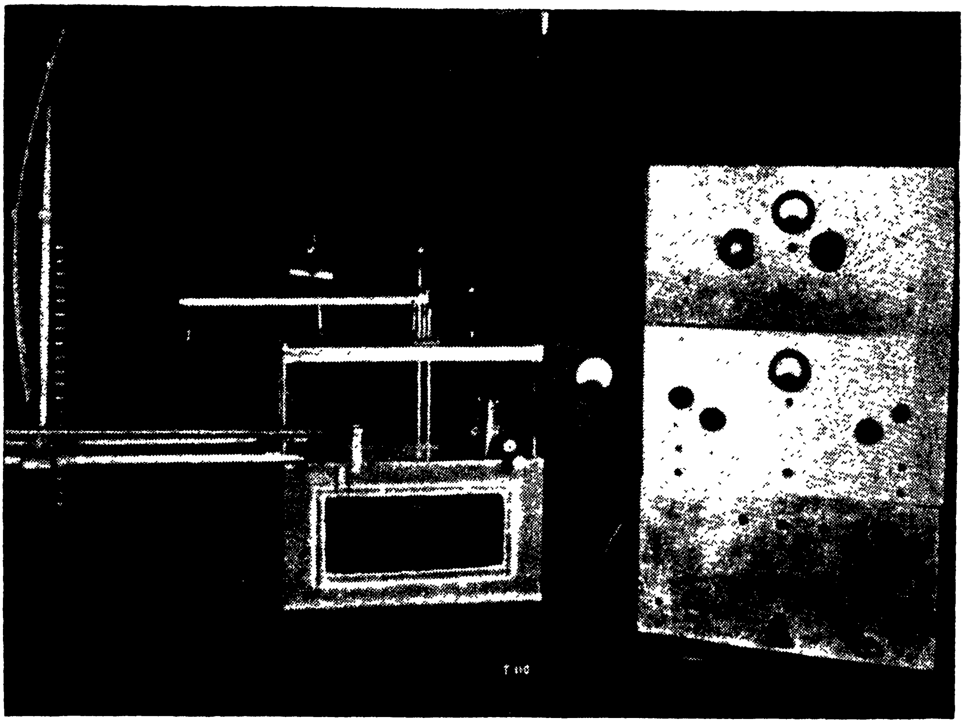

FIG. 2*a*. Photograph shows the arrangements of the experimental set-up.

(*b*) *Source of Ultrasonic Energy*

An assembly of an X-cut piezoelectric quartz plate having 3·0 cm. in diameter and 0·287 cm. in thickness and the electrode systems for feeding electrical power into the crystal was used as the transducer. The crystal plate had a silver plated area of 3·8 sq. cm. It was housed in a small brass-cartridge (Fig. 2). One plated face was in contact with the brass-cartridge and also exposed to the liquid under investigation. The other face was insulated from the housing body but was backed by air. By means of a co-axial cable, the transducer was connected to the r.f. voltage source. The co-axial cable was sealed against ingress of the liquid being treated by a rubber packing ring. To give maximum power output in the forward direction, the generating crystal was backed by a specially designed air-chamber. Careful design of the rear connection arrangement to the crystal eliminated the possibility of corona discharge at the high operating r.f. potential. Higher harmonic of the crystal was used in the present investigation. The operating frequency was 11 Mc/s. The potential that was applied to the transducer was about 100 volts.

(*c*) *Cavity Radiometer*

An acoustic 'hohlraum' is a practicable approach to a perfect absorber. It is a cavity with acoustically insulating walls, filled with an absorbing liquid, and provided with a window through which the acoustic beam is admitted. This device is to replace the conventional absorbing or reflecting solid surface in the measurement of radiation pressure. It consists of glass tube—the cross-section of which is larger than the cross-section of the acoustic beam. The length of the tube is so adjusted that at the operating frequency of the order of megacycles, the absorption of energy is complete in the tube. The remote end of the tube is closed by an absorbing material such as rubber. The plane of the window, therefore, serves as a totally absorbing surface. Fig. 2 shows the schematic diagram of the cavity radiometer.

The cavity *C* was so adjusted that its axis was along the centre of the beam. The orientations of the transducer were adjusted until the beam entered the centre of the tube. This was done by using a dye solution from a fine capillary tube into the beam but close to the centre of the quartz crystal; the dye was carried along the streamline. By observing these streamlines, the final adjustments were made.

The length of the tube is 13 cm. and its inner diameter is 3·8 cm. The other end of the tube is closed by a clean rubber which acts as an excellent absorber at frequencies above 10 Mc/s.

(*d*) *Electrical Circuits*

The electrical circuit arrangements are shown by block diagrams in Fig. 3.

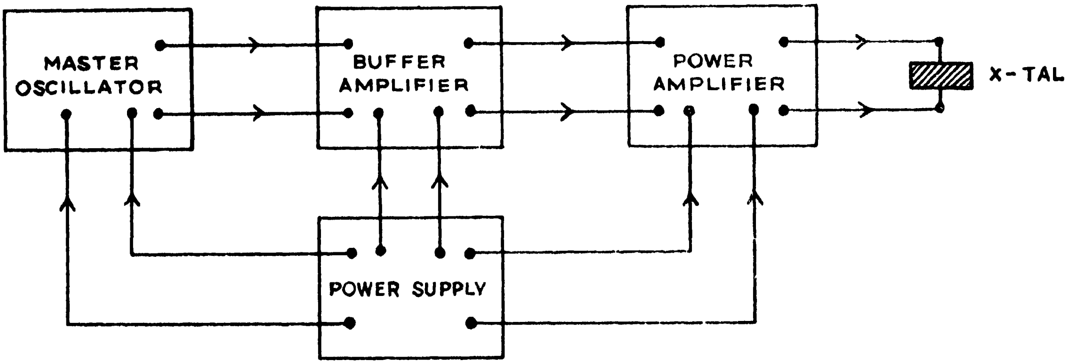

FIG. 3, BLOCK DIAGRAM OF ELECTRICAL CIRCUITS

The circuits of the different units are of conventional types, and so no details are incorporated here. R.F. power from the amplifier was delivered to the crystal transducer through a co-axial cable which was matched to the system by trial and error method. L/C ratio of the tank coil across which the transducer was connected was made high, so as to get a sharp resonance peak. Under this condition, a slight movement of the variable condenser of the tank circuit caused the transducer to vibrate at a frequency far off from the resonant frequency. This was indicated by a r.f. meter inserted in series with the transducer. As a check to the constancy of frequency during the time of experiment, a wave meter was placed nearby the tank circuit. Any change of frequency was indicated in the meter incorporated in the wave meter.

The amplifier can supply r.f. voltage of about 100 volts (variable) at 11 Mc/s. which is the 11th harmonic mode of vibration of the transducer.

4. EXPERIMENTS

Alignments and levelling of the different mechanical and optical parts of the apparatus are of crucial importance in this experiment. To ensure better accuracy of results, it is essential that the entire cross-section of the acoustic beam should enter the cavity radiometer and be normal to the far end terminating material of the cavity radiometer tube at all distances from the source of acoustic energy. This state of condition was studied by means of (i) dye solution which made the streamlines visible, (ii) probing disc provided with a pointer which grazed along the surface of a scale. These two devices acted as indicators of the state of acoustic beam, i.e. the streamline flow. Actual alignments were made by adjusting the baffle containing the transducer. This baffle has got provisions for all sorts of motions, i.e. (i) lateral, (ii) up and down and (iii) rotational motion about the horizontal and the vertical axes.

The above-mentioned adjustment of the apparatus being made, experiments on the measurement of the forces acting on the detecting element were carried out under following two heads:

(i) Measurements of the force imparted on the detecting element due to radiation only at different distances from the source of acoustic energy.

(ii) Measurement of the force imparted on the detecting element due to streaming only at different distances from the source.

5. OBSERVATIONS AND RESULTS

The sensitivity of the measuring system is such that it produces a deflection of 1 mm. on a scale placed at a distance of 1 metre from the mirror for a change of mass of 2 mgm. in the scale-pan.

Fig. 5 shows a photograph of the streamline made visible by dye solution.

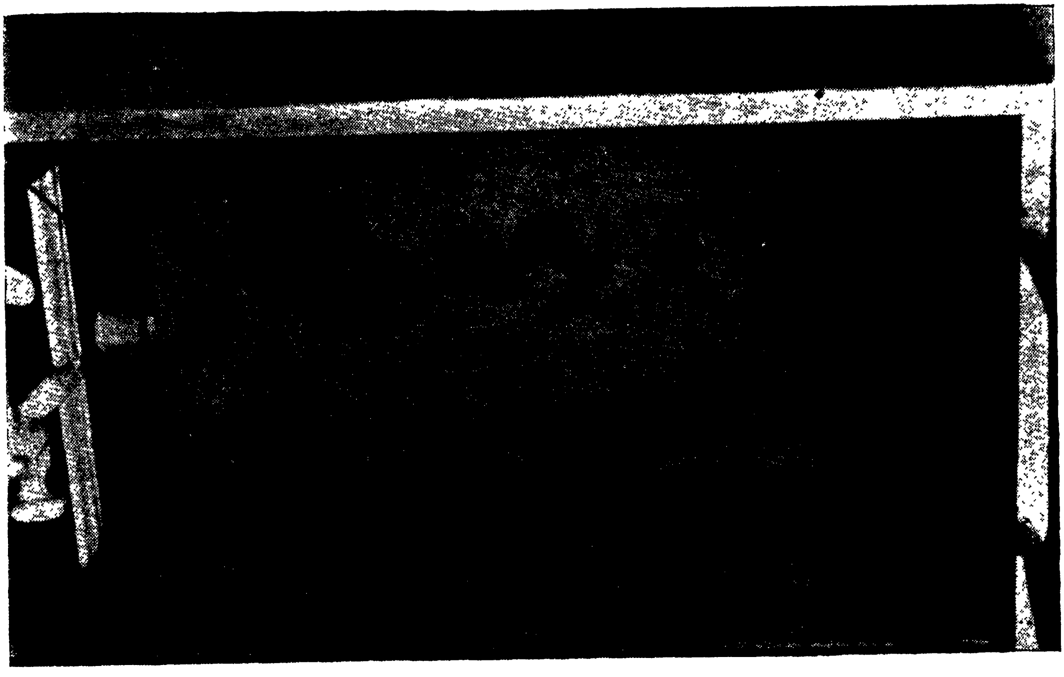

FIG. 5. Photograph shows the streamline pattern. Irregularity occurring at about 8 cm. (*p*) from the source (*s*). Scale: 1 cm. ≈ 2·8 cm. (approximately).

It is apparent from the photograph that the thin steam of coloured liquid flowed for a distance of about 8 cm., and afterwards it became irregular and diffuse. This corresponds to the similar irregularity obtained in the streaming pressure measurements.

The summarized results of the present investigations are shown in Table I and part of the results is shown by means of graphs in Fig. 4*a* and the results obtained by Cady and Gittings are shown in Fig. 4*b* for a comparative study. Cady and Gittings' results show that up to a distance of 13 cm. the mean force was 42·9 dynes, the largest deviation being about one per cent. In the present investigation, by extrapolation the energy density at the transducer was found to be 68·3 ergs/cm.^{3} (68·3 dynes/cm.^{2}).

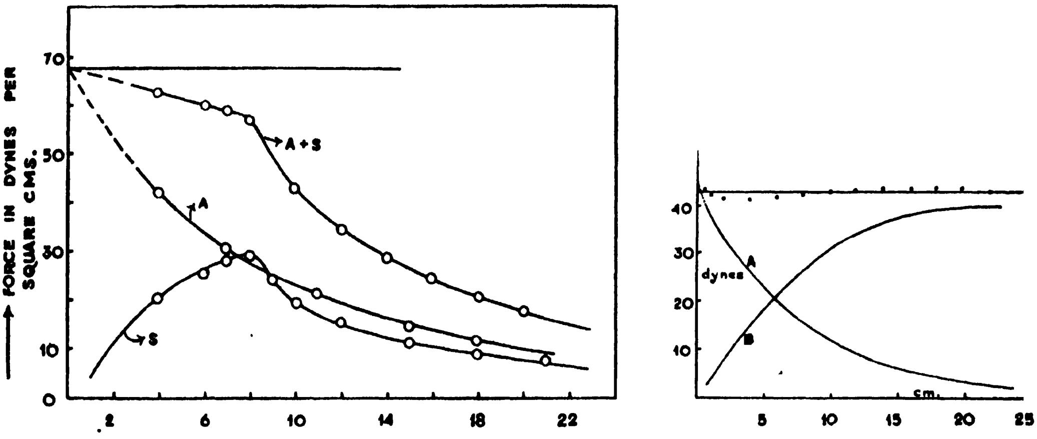

FIG: 4, A REPRESENTS FORCE DUE TO RADIATION. S THAT DUE TO STREAMING. AND A+S REPRESENTS THE SUM OF RADIATION AND STREAMING FORCES. DRIVING VOLTAGE 100.

(*a*)

A, FORCE ON TUBE ABSORBER WITH MEMBRANE 3 CM IN FRONT OF IT. B, FORCE ON MEMBRANE ALONE. ABSCISSAS ARE DISTANCES FROM TRANSDUCER TO MEMBRANE. DRIVING VOLTAGE 70.

(*b*)

Up to a distance of about 8 cm. from the source, the total force decreased at a very slow rate. The intensity of acoustic energy decreased exponentially with distance. It is shown in Fig. 4, curve *A*. The intensity of the force due to streaming increased with distance up to a distance of about 8 cm. from the source. Afterwards, it decreased. The result obtained is shown in Fig. 4, curve *S*. While measuring acoustic force, a collodion membrane was placed at a fixed distance of 1 cm. from the absorber *C*, as shown in Fig. 2, B_3. While measuring the streaming force, the membrane was removed from the baffle B_3 and was fixed at the window in the baffle B_2. This time the rubber block closing the far end of the cavity tube was removed. Fig. 1 shows the principle of the mechanism of measuring the acoustic and the streaming forces. The diagram itself is self-explanatory.

The acoustic force was measured at different places from the source. The summarized results are shown in Fig. 4, curve *A* and the corresponding results for the streaming force are shown in curve *S*. Curve $A+S$ shows the sum of the ordinates in curve *A* and curve *S*.

By applying the Newton's law, acoustic energy density was measured by a single measurement. In this case, feeding the r.f. voltage to the transducer was found to be a hard task. Connecting wire reduced the free movement of the measuring system. Anyhow, this trouble was reduced to minimum by trial and error process. The measure of the acoustic energy density as determined by this method was found to be 64 ergs/cm.^{3},

Experimental data:

TABLE I

S. No.	Pressure due to	Distance of the baffle B_3 from the source in cm.	Total average force in mgm. wt. and max. possible error in each reading in mgm. wt.	Force per unit area dynes/cm.^{2} and max. possible error in each reading in dynes/cm.^{2}	From Radiation Pressure Measurements	By measuring the reacting force on the transducer	$\alpha/f^2\times10^{14}$ db/m. sec.^{2} at 29°-30°C. (by author) for water at 11 Mc/s. and driving voltage = 100 v.	$\alpha/f^2\times10^{14}$ db/m. sec.^{2} at 30°C. (by Pinkerton) for water	$\alpha/f^2\times10^{14}$ db/m. sec.^{2} at 30°C. calculated for water
1	2	3	4	5	6	7	8	9	10
1.	Acoustic Radiation	4	165 ± 2	42·6 ± 0·5	68·3 Ergs/cm.^{3}	64·0 Ergs/cm.^{3}	14·3	16·6	5·5
		7	120 ± 2	31·0 ± 0·5					
		11	85 ± 2	21·9 ± 0·5					
		15	57 ± 2	14·7 ± 0·5					
		18	45 ± 2	11·6 ± 0·5					
2.	Streaming	4	80 ± 2	20·6 ± 0·5					
		6	100 ± 1	25·8 ± 0·25					
		7	112 ± 1	28·9 ± 0·25					
		8	115 ± 1	29·7 ± 0·25					
		9	95 ± 2	24·5 ± 0·5					
		10	75 ± 2	19·4 ± 0·5					
		12	60 ± 2	15·5 ± 0·5					
		15	45 ± 2	11·6 ± 0·5					
		18	35 ± 2	9·0 ± 0·5					
		21	30 ± 1	7·7 ± 0·5					

Table I shows the summarized results of the present investigations. Figures after the sign ± in columns 4 and 5 indicate the maximum possible error that may creep in each reading. Columns 6 and 7 show the values of output acoustic energy as obtained (i) from radiation pressure measurements and (ii) by measuring the reacting force on the transducer respectively. Column 8 shows the value of $\alpha/f^2\times10^{14}$ db/m. sec.^{2} as obtained by the author and column 9 that as obtained by Pinkerton. Column 10 shows the theoretical value based on shear viscosity.

The attenuation coefficient as estimated from the curve A, by using a relation like $P_x = P_0 e^{-\alpha x}$, where α is the attenuation constant, P_0 and P_x are the pressures at the positions separated by x units of length. Value of α as obtained in present experiment for distilled water is 17·35 db/m at 29°C.–30°C. and $\alpha/f^2 = 14{\cdot}3 \times 10^{-14}$ db/m sec.^{2}.

6. Discussions

The results of the present investigations show that the intensity of acoustic force decreases exponentially with distance whereas that of streaming force, after increasing up to a distance of about 8 cm., decreases exponentially. The sum of these two forces (acoustic and streaming), obtained by adding the magnitudes of the separate forces at the same place, is found to decrease at a very slow rate up to a distance of about 8 cm., and then decreases exponentially. Photograph of the streamlines also shows same irregularity in the streamline pattern after about 8 cm. The analysis of these results reveals that up to a distance of 8 cm. most of the acoustic energy is converted into streaming, i.e. mass flow, and only a small fraction is lost due to other causes. The exponential decrease after this region may be supposed to be due to formation of some sort of turbulent motion which is also evident from the photograph. At this region, the velocity of streaming might have reached the extent of setting up turbulent motion. At larger velocity there appear irregular deviations that have a tendency to fill the whole vessel. The initially straight streamline became fuzzy and finally indistinguishable after about 8 cm. in these investigations. Up to 8 cm. the motion is laminar type (proceeding in regular laminations as in the Hagen-Poisseuille law), after this region the motion is turbulent type.

Viscosity tends to play the part of smoothing out lateral components either initially present or produced by roughness of the vessel; it thus favours laminar pattern. The inertia of the fluid requires the conservation of such side components once they have come into existence; thus it acts in favour of turbulence. The antagonism between viscosity and inertia that becomes apparent even in the structure of the expression $\nu = \mu/\rho$ which occurs in the denominator of the expression for the Reynold's number $R = \frac{Va}{\nu}$. Increasing ρ diminishes ν and favours turbulence. (All the symbols have the usual meaning.) The above arguments throw light on the cause of the formation of turbulent motion after about 8 cm. in the present investigation.

Cady and Gittings also did observe irregularity in the result of a typical series of observations. Up to 13 cm. the mean force on the absorber was found almost constant. From 13 cm. on, the values became irregular. The dye injected into the beam close to the transducer flowed uniformly for distance of 12 cm. and from there on it gradually became irregular and diffuse, indicating similar irregularity in the hydrodynamic flow. This state of affairs happened in the present investigation a little earlier, at about 8 cm. Cady and Gittings carried out the experiment at 15 Mc/s, with driving voltage 70, whereas the present investigation was carried out at 11 Mc/s, driving voltage 100.

According to Borgnis, the sum of the radiation and the streaming pressures should remain constant over a considerable distance except being modified by some other forces of small magnitude being generated in the system. Cady and Gittings' result, which is shown in Fig. 4, satisfactorily verified the Borgnis' postulate up to a distance of about 25 cm. But this constancy was not observed in the present investigation up to the extent as observed by them. An interpretation of the observed anomaly in the present investigation has been given in the preceding paragraphs. According to the experimental results of Cady and Gittings, whole amount of acoustic energy is converted into streaming and up to a distance of about 25 cm. (from graph) viscosity of the medium plays no part in dissipating acoustic energy. This is something unusual. For clear understanding of the anomaly, further work under varied conditions is necessary.

But in an experiment, Cady and Gittings (1953, *J. A. S. A.*, p. 896, Fig. 6, Curve *C*) observed deviations from constant value and attributed the cause to be due to a violation of the boundary conditions.

The attenuation constant α as obtained from the measurements of radiation pressure at different distances from the source comes out to be 17·35 db/m at 29°-30°C. and $\alpha/f^2 = 14{\cdot}3\times10^{-14}$ db/m sec.^{2} at $f = 11$ Mc/s. as against $16{\cdot}59\times10^{-14}$ db/m sec.^{2} at 30°C. as obtained by Pinkerton (1947). The value of α/f^2 as obtained in the present investigation approaches nearer to the theoretical value based on shear viscosity, but agrees fairly well with that obtained by Pinkerton.

It has been observed that the proper choice of experimental tank or vessel in which an experiment is carried out plays an important rôle. Depending upon the condition of the side walls and end walls of the experimental tank, the acoustic energy field pattern gets deformed and so any measurement made in that condition may not yield the correct information. Under these circumstances, it is essential that measurements by the different methods be carried out under identical conditions of the tanks so that comparable results are obtained to establish complete information on all the relevant factors which provide the better background for a theoretical understanding of the absorption problem.

7. ACKNOWLEDGEMENT

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XI. PART II. ON THE MEASUREMENT OF ACOUSTIC ENERGY DENSITY AND STREAMING VELOCITY TO DETERMINE THE ABSORPTION COEFFICIENT BY THE STREAMING METHOD * †

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(*Received for publication on January 3, 1958*)

Abstract

Acoustic energy density at a fixed distance from the source of energy has been measured by radiation pressure method.

An apparatus has been designed in such a way as to translate Eckart's theoretical idea of streaming into practice. The annular space between two co-axial tubes has been made the quiescent return path of streaming as required by Eckart's theory.

A mathematical expression has been derived for the maximum velocity of streaming through the annular space.

The Poisseuille's formula has been modified so as to fit the case of streaming through the annular space in order to determine the absorption coefficient.

Streaming velocity is found to be proportional to absorption coefficient.

Absorption coefficients thus determined for water, methyl alcohol and ethyl alcohol approach nearer the theoretical values compared to those obtained by other authors. No such measurements were possible with the three highly absorbing liquids—carbon disulphide, benzene and carbon tetrachloride.

1. Introduction

It is a well-known fact that the absorption of ultrasonic energy in liquids exceeds by far the absorption predicted by the classical theory based on viscosity. In order to resolve the existing large discrepancy between the theory and the experiment, various theories have been put forward to include factors supposed to be responsible for the high absorption but still no satisfactory explanation has yet emerged. For very extended literature, reference may be made to the detailed bibliography contained in the following reviews (1) by W. T. Richardson—*Rev. Mod. Phys.*, **11**, 36 (1936); (2) by Markham, Bayer and Lindsay—*Rev. Mod. Phys.*, **23**, 353 (1951). Until recently, this absorption has been attributed mainly to (1) viscosity, (2) heat conduction, and (3) head radiation. The success achieved, thereby, is very little. So in the next phase, the directions of endeavours have been oriented and focused on (1) 'The relaxation theory', (2) 'The molecular structure theory', and (3) 'The second viscosity coefficient theory'.

(i) The relaxation theory has to a great extent resolved the previous discrepancy between calculated and experimental values of ultrasonic absorption in gases. This theory was developed by many workers, who put forward modified forms of the theory of liquids. Basic hypothesis behind this theory is that a much larger number of collisions is required to achieve thermal equilibrium for molecular vibrations than for the translational and rotational vibrations. So there is a time lag in the establishment of equilibrium for the internal degrees of freedom, and hence, there must be dissipation of energy.

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* This paper contains the essential results of a thesis approved by the Calcutta University for the degree of Doctor of Philosophy (1957).

† Part of the results reported to Ann. Rept. Bose Institute, 1955-56.

(ii) The molecular structure theory, as proposed by Hall (1948), is of recent origin. This theory searched for the excess ultrasonic absorption in water, not in the time lag for the establishment of the equilibrium of energy distribution among the different internal degrees of freedom but in the lag of the rearrangement of the molecules during the acoustic compression. The passage of acoustic energy changes the equilibrium distribution of molecules; (i) in a compression, molecules from the open structure may move to the closely packed one, and (ii) in a rarefaction from the closely packed structure to open one. In order to give effect to a change from one state to another, some work has to be done. The coefficient of viscosity necessary for this is almost equal to that necessitated for the viscous flow. The associated relaxation time for this process has been found to be 10^{-12} sec. for water. This mechanism hypothetically introduces a bulk viscosity coefficient (K) or a second viscosity coefficient (μ') for water, whose measure is several times the magnitude of shear viscosity for water. The excess absorption thus calculated for water has been found to agree with the observed value so excellently as to leave no doubt that at any rate for water the problem has been solved.

The introduction of these factors although seems possible to give full account of the dissipation mechanism of ultrasonic energy in liquids, yet it is too early a stage to reach a final decision.

(iii) The term 'second viscosity' is not a familiar one. The classical theory of viscosity is very similar to the classical theory of elasticity. In elasticity it is necessary to introduce two moduli of elasticity; similarly it might have been expected that in order to describe the dynamics of fluid motion it would be necessary to introduce a shear viscosity and some 'second viscosity'. The term viscosity suggests internal friction in fluid media and the coefficients of viscosity generally measured and given in tables are shear viscosity. When a volume of liquid is compressed without change of shape, viscous forces come into play. Some of these may be due to viscous forces of the shear type, but some of them may be forces of the 'second viscosity type'. It is evident that the amount of work done by the viscous forces depend upon the rate of compression. No direct determination of the coefficient of second viscosity has yet been made, because no direct method is available for determining it. Recently, however, a determination has been made by evaluating the ratio of the coefficient of the second viscosity of shear viscosity, when the ultrasonic vibrations are passed through the liquid (Liebermann, 1949).

The idea of introducing a term 'second viscosity coefficient' to resolve the existing discrepancy between the theoretical and experimental values of the absorption coefficient of ultrasonic waves in liquids is of recent origin. It is the outcome of the reconsideration of the hydrodynamic equations. The classical theory of dynamics of viscous fluids is based on the assumption that there is only one fundamental coefficient of viscosity —μ, the coefficient of shear viscosity. The other μ', the second viscosity coefficient, is assumed to be equal to $-2/3\mu$, in order that $K=(2\mu+3\mu')/3$, the coefficient of bulk viscosity, should be zero. In making this assumption, classical hydrodynamics parts company from classical elasticity, in which two fundamental quantities, the Lame' constant μ and λ are introduced. Stokes, neglecting the second viscosity coefficient but taking into account the internal friction of the sound conducting medium, derived an expression for the absorption coefficient of acoustic waves in liquids.

At low frequencies in the sonic range, the compressions and rarefactions in liquids are not of considerable importance in connection with the absorption of energy. But at ultrasonic frequencies, even liquids may undergo compressions and rarefactions involving considerable absorption of energy. Accordingly, Tisza (1942), Eckart (1948), Markham (1952) and others (for comprehensive references see *Proc. Roy. Soc.*, Sec. A, Vol. 226, October, 1954) re-investigated the hydrodynamical equations of motions in the above light.

Tisza introducing the bulk viscosity coefficient developed a quantitative theory of the absorption of sound in liquids.

Eckart developed a general formalism for solving sonic flow problems and applied it to the case of streaming. He showed that the streaming speed in any fluid depends on the bulk viscosity (second viscosity) coefficient μ' for that liquid and derived an expression connecting μ and μ', capable of experimental test.

This idea of Eckart was translated into practice by Liebermann (1949) by dint of his ingenious experiment. He calculated the coefficient of bulk viscosity for various liquids, using Eckart's theory.

In the hands of Markham, Eckart's theory was further extended by introducing an equation of state of a more general one which takes into account the relaxation effects. This leads to the fact that contribution of relaxation process to the streaming is of the same order of magnitude as that might be ascribed to bulk viscosity.

Nyborg (1953) has shown that streaming speed depends critically upon α, the attenuation constant, where α may be due to any mechanism whatsoever, such as heat conduction, scattering, thermal relaxation, etc. From these results, he concluded that streaming measurements cannot be used to distinguish between absorption mechanisms.

Experimental verification of Nyborg's postulate was done by Piercy and Lamb (1954). They determined with the help of their apparatus the sound intensity and the streaming velocity. These results were used in the Poisseuille's formula for flow velocity and from which the absorption coefficients of ultrasonic waves in liquids were determined.

The present investigation is an attempt to measure the acoustic energy density by radiation pressure method and hence to determine the absorption coefficient by the streaming method. In order to determine the absorption coefficient by this method, the Poisseuille's formula which has been modified to fit the case of streaming through the annular space between two co-axial tubes is used. This annular space serves the function of the quiescent return path for the flow of liquid as pointed out by Eckart's theory. Measurement of acoustic energy density by the device adopted in the present investigation seems most effective in cases like the present one. This measurement by other methods as employed by Bhadra (1953, 1955) does not seem promising and advantageous in consideration of the size of the liquid container and the quantity of liquid required. The design of the apparatus used in the present investigation has been made in such a way that no other forces save the progressive radiation force can interact with the radiation pressure detecting disc. Provision has also been made to cut the effect of streaming on the disc while measuring the acoustic energy density.

Streaming velocity is generally recorded by the transit time of the particles, remaining in suspension in liquid, taken to travel a known distance. These particles are not visible to naked eye but they are made visible in order to gain the objective by illuminating them with an external source of light. Due to the viscosity of liquids, different particles move with different velocities through the annular space depending on the pressure gradient along the radius of the tube. So, to avoid confusion, maximum velocity of streaming is determined and accordingly an expression for the maximum velocity through the annular space is developed. Maximum velocity corresponds to the minimum transit time.

From the experimentally determined values of E_x, the acoustic energy density, $v(r)$ max, the maximum streaming velocity, which is a function of the radial distance from the axis, the absorption coefficient, α, is determined. The values of α, as determined for water, methyl alcohol (analar quality, acetone free, Merck) and ethyl alcohol (spec. quality), approach nearer to theoretical values comparable to those obtained by other authors. But in the case of the three highly absorbing liquids, i.e. carbon disulphide, benzene, and carbon tetrachloride, E_x and $v(r)$ max could not be determined as the indicator did not show any change. Further investigations on the last named liquids are kept in abeyance till a suitable experimental arrangement is made befitting the situation to study the same.

2. CALCULATIONS OF STREAMING AND ABSORPTION COEFFICIENT

Flow of acoustic energy through an attenuating liquid is associated with interference and divergence of sounds. So the time constant terms in radiation pressure P depends on position. Thus there will be a constant pressure gradient, which will produce a constant acceleration of the fluid. The velocity of the fluid will increase until the viscous forces balance the pressure gradient, and resulting thereby steady-state streaming. For the production of acceleration of the fluid, it is necessary that there exists a relatively quiescent return path. This return path is assumed to be the annular space between two co-axial tubes. The cross-section of the inner tube is such that the acoustic beam completely fills the tube. The area of the cross-section of the annular space is approximately equal to that of the inner tube. This condition reduces the diminution of the steady-state velocity of streaming, i.e. the resistance to flow.

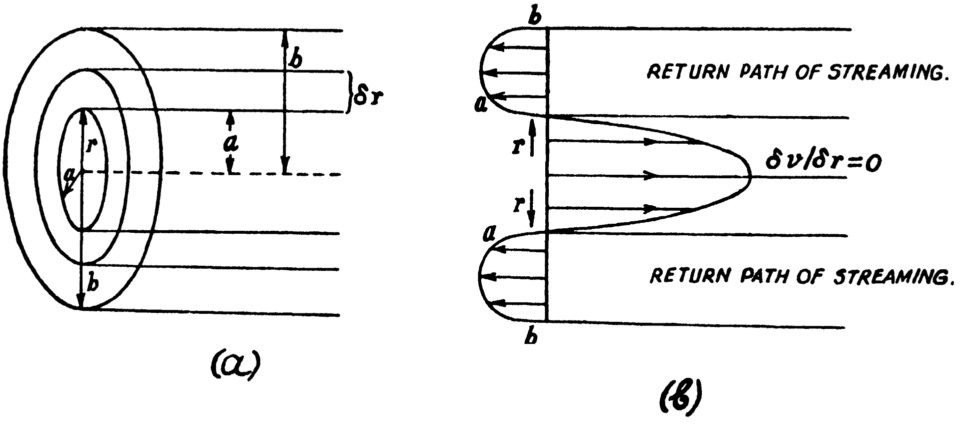

FIG. 1. (*a*) shows the cross-section of the tubes, and (*b*) shows the velocity profile.

Let $v(r)$ be the velocity of the fluid, everywhere being parallel to the direction of flow (Fig. 1). The reactions due to viscous friction are proportional to the velocity gradient, the factor of proportionality η depending on the nature of the fluid. The reactive forces are referred to as frictional or viscous forces.

$$F = -\eta A.\frac{dv}{dr} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where A is the area over which the forces act.

Let $\frac{dF}{dr}$ express the rate of change of this force with r, then the value of the difference between the magnitudes at the two surfaces of the tube, since the thickness is δr, is $\frac{dF}{dr}\delta r$.

In the case of the steady flow we have, therefore, the equal and opposite forces, which may be expressed as

$$-\left(\frac{dF}{dr}.\delta r\right) = 2\pi rP.\delta r,$$

where P is the difference of pressure between the ends of the tube,

or
$$-\frac{dF}{dr} = 2\pi rP \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The viscous force is given by $F = \eta 2\pi rl\frac{dv(r)}{dr}$ .. .. (3)

where v is the velocity of streaming, v depends on r, i.e. $v(r) = f(r)$.
Equation (2) becomes

$$\frac{d}{dr}\left(-\eta\frac{dv(r)}{dr}.2\pi r.l\right) = 2\pi rP$$

or

$$\frac{d}{dr}\left(r\frac{dv(r)}{dr}\right) = \frac{rP}{\eta l}$$

Integrating,

$$r.\frac{dv(r)}{dr} = -\frac{Pr^2}{2\eta l} + C$$

Again integrating,

$$v(r) = -\frac{Pr^2}{4\eta l} + C\log r + D \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where C and D are constants of integration. Now applying the boundary conditions we get

when
$$\left.\begin{array}{l} r = a,\ v_a(r) = 0 \\ r = b,\ v_b(r) = 0 \end{array}\right\} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where $2a$ represents the outside diameter of the inner tube and $2b$, the inside diameter of the outer tube. From equations (4) and (5) we get

$$\left.\begin{array}{l} O = v_a = \frac{-P_a 2}{4\eta l} + C\log a + D \\ O = v_b = \frac{-P_b 2}{4\eta l} + C\log a + D \end{array}\right\} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

From (6) we get
$$C=\frac{P(a^2-b^2)}{4\log a/b} \quad \cdots \quad (7)$$

Substituting the value of C in (6), we get
$$D=\frac{P.b^2}{4\eta l}-\log b.\frac{P}{4\eta l}\cdot\frac{a^2-b^2}{\log a/b} \quad \cdots \quad (8)$$

$$\text{Put } \epsilon=\frac{P}{4\eta l}$$

From equations (4), (7) and (8), we get
$$v(r)=\epsilon\left[(b^2-r^2)+\frac{a^2-b^2}{\log a/b}\cdot\ \log r-\frac{a^2-b^2}{\log a/b}\cdot\ b\right] \quad \cdots \quad (9)$$

$$\frac{dv(r)}{dr}=-2\epsilon r+\frac{\epsilon}{r}\cdot\frac{a^2-b^2}{\log a/b} \quad \cdots \quad (9a)$$

When $v(r)$ is maximum, $\frac{dv(r)}{dr}=0$.

From (9) we get, when $v(r)$ is maximum, $r=[(a^2-b^2)/2\log a/b]^{\frac{1}{2}}$ .. .. .. (10)

Substituting this value of r in equation (9), we get
$$v(r)_{\max}=\epsilon\left[\left(b^2-\frac{a^2-b^2}{2\log a/b}-\frac{(a^2-b^2)}{\log a/b}\right)\cdot\ \log b+\frac{a^2-b^2}{2\log a/b}\cdot\right.$$
$$\left.\log\left(\frac{a^2-b^2}{2\log a/b}\right)\right]=\frac{P}{4\eta l}\cdot X(r) \quad \cdots \quad (11)$$

where $X(r)$ stands for the expression within the square brackets in equation (9) and this is the constant of the apparatus.

In equation (11), P stands for the pressure difference between the ends of the inner tube of length l units. The pressure difference is due to the flow of acoustic energy through the tube. The time independent gradient of pressure that exists along the axis of the tube causes the streaming. Inside the inner tube both the radiation and the streaming flow. The streaming returns through the annular space between the two co-axial tubes. There is one directional flow through the inner tube as well as through the annular space. Therefore, the pressure difference P may be taken to be equal to the energy density difference of acoustic waves.

Energy density at a distance x from the source, when acoustic wave is propagated along the axis of the tube, is given by
$$E_x=E_0(r)e^{-\alpha x}, \quad \cdots \quad (12)$$

where α stands for the absorption coefficient associated with the excess pressure variation in the sound wave. If the exponent is sufficiently small, equation (12) can be approximated by
$$E_x=E_0(r)[1-\alpha x]. \quad \cdots \quad (13)$$

The driving force Y, responsible for the acoustic process, is given by
$$Y=-\frac{\partial P}{\partial x}=\alpha E_0(r). \quad \cdots \quad (14)$$

The radiation pressure is a force per unit area acting in the direction of propagation of the sound wave of magnitude equal to energy density E_x.

So P may be replaced by ΔE, i.e. the difference in energy density at the two ends of the inner tube, and it may be expressed in the following way:

$$\Delta P = \Delta E = E_x \alpha l \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (15)$$

where l stands for the length of the inner tube and E_x represents the energy density at a distance x from the source.

Now the Poisseuille's formula, as modified to fit the case of the streaming of liquids through the annular space between two co-axial tubes, may be expressed in the following way by combining equations (11) and (14)

$$v(r) \max = \frac{E_x \alpha}{4\eta} X(r). \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (16)$$

From equation (16) it is clear that by determining E_x, and $v(r)$ max. experimentally and taking the value of η, the coefficient of shear viscosity from I.C.T., the value of α, the absorption coefficient can be determined.

3. APPARATUS FOR MEASURING ACOUSTIC ENERGY DENSITY AND STREAMING IN LIQUIDS

The physical nature of the approach to streaming based on the gradient of radiation pressure has been found to be more applicable to experiment than the mathematical approach of the theoretical investigators. The experimental arrangement that has been adopted for the measurement of the ultrasonic intensity and streaming in liquids at frequencies about 11 Mc/s is shown in Fig. 2.

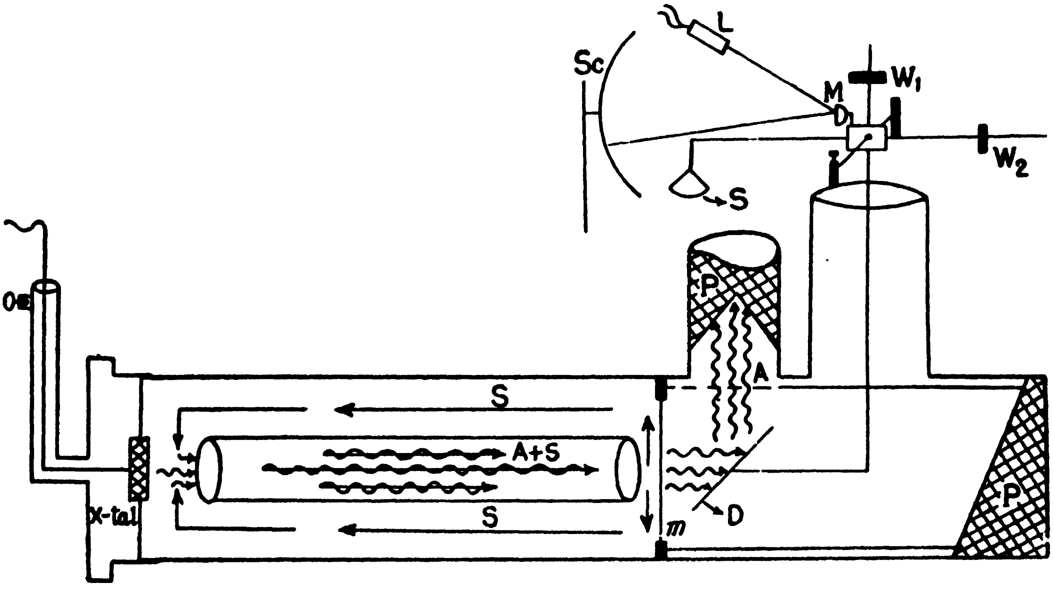

FIG. 2. Shows the schematic arrangement of the apparatus and the physical aspect of the process. *X-tal*—stands for crystal transducer; *S*—streaming (⟶); *A*—acoustic radiation (〰→); *A* and *S*—radiation and streaming (〰→); *m*—screen; •*D*—detecting element—a polished disc; *P*—polystyrene (acoustic energy absorber); *M*—mirror; *Sc*—scale; *L*—lamp; W_1 and W_2—weights to adjust sensitivity and balance of the system.

The ultrasonic beam completely fills the inner tube and does not extend into the annular space between the two co-axial tubes. This annular space provides a return path for the streaming. The cross-sectional areas of the main tube and that of annular space are made approximately equal in order to reduce the resistance of the flow of liquid. Fig. 2, which shows the schematic arrangement of the apparatus, itself explains the functional principles involved. The crystal transducer *X*-tal sends a beam of progressive ultrasonic waves at 11 Mc/s which completely fills the inner tube as shown schematically by straight lines and undulatory lines representing respectively streaming and radiation. The thin cellophane screen *m* which is placed in close proximity to the disc in the path of the beam stops the streaming but allows the flow of radiation practically undiminished. This screen *m* acts as an acoustic bypass. Radiation after crossing the screen impinges on the disc and transferring momentum on it gets reflected in the direction, as shown in the diagram, where it is absorbed by polystyrene *P*. Due to this transfer of momentum, the rod *R* suffers a rotation, which is observed by the deflection of a light spot on a scale. The highly polished disc *D* is fixed to the rod *R* being inclined at an angle of 45° to the axis of the tube, which is also the axis of the acoustic beam. This inclination of the disc provides a means of deviating the acoustic beam in a direction at right angles to the direction of the incident beam so as to avoid interference between the incident and the reflected beam, and thus getting rid of the formation of stationary waves in front of the disc. The cross-bar had additional provisions for holding a scale-pan and a balancing adjustment system. By adding known weights on the scale-pan, the light spot is brought back to the original position. The weight required gives the measure of the ultrasonic energy density, at the place. The distance between the source of ultrasonic energy and the centre of the disc is 17 cm. The sensitivity of the apparatus is such that a force of

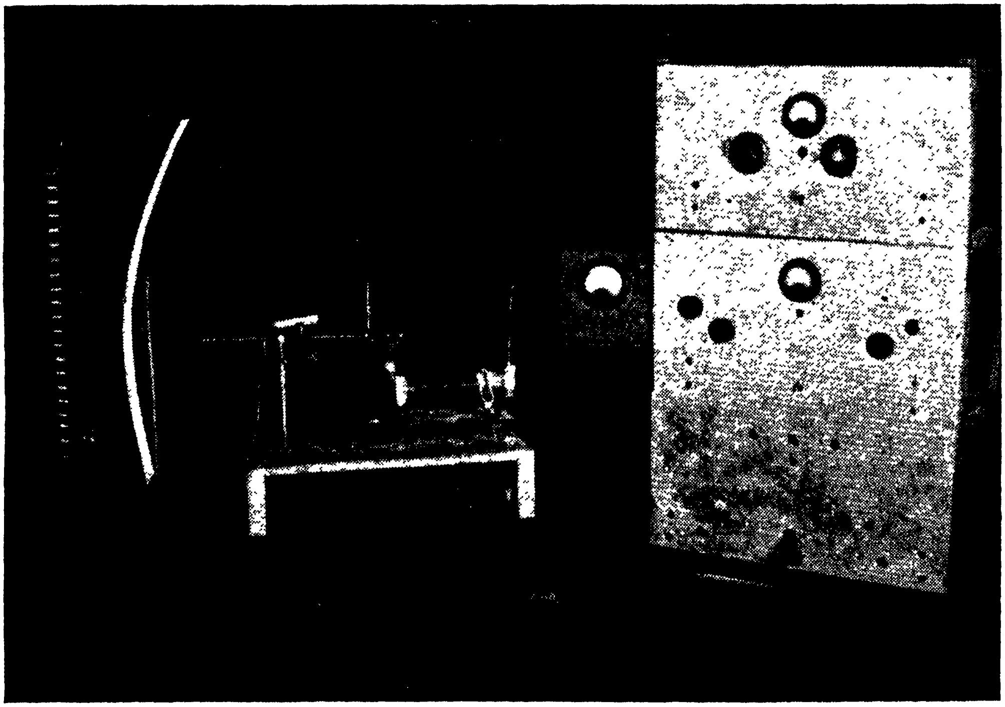

FIG. 3. Photograph shows the experimental arrangement of the apparatus.

1 mgm. wt. can be determined. The maximum velocity of streaming, which is a function of the radius of the tube, is determined by recording the transit time required by particles present in suspension in liquid to cover a fixed distance. Ordinarily the particles are not visible to naked eye. But when the liquid is illuminated externally by some moderately strong source, these particles become visible, and under such condition, the determinations of the transit time of the particles are done effectively.

Fig. 3 shows the photograph of the experimental arrangement of the apparatus.

With the apparatus just described, the intensity of ultrasonic energy at a fixed distance from the source and the maximum streaming velocity can be determined with greater accuracy. Radiation pressure detecting disc is kept in a position under such conditions that only the radiation interacts with the disc. Necessary precautions are undertaken in the design of the apparatus to see that no other factors come into play. If there be some unavoidable factors still present, the effect due to these factors must be of very small magnitude, which can be easily rounded off in comparison with the effect produced due to radiation without introducing much error in the results.

4. EXPERIMENTAL DETERMINATION OF ACOUSTIC ENERGY DENSITY

Acoustic energy density at 17 cm. away from the face of the crystal transducer was determined by radiation pressure method. Necessary precautions were undertaken in the design of the apparatus to see that the radiation pressure detecting disc was not interacted upon with forces other than the radiation force. Here the disc acted as a perfect reflector. Due to the interaction of the radiation with the disc, a torque was produced on the cross-bar. This effect was observed by means of the deflection of the light spot on a scale. By adding known weights on the scale-pan, the excess pressure was measured directly. Numerical value of this measured excess pressure gave the measure of the energy density. Maximum possible error in pressure measurement was ± 0·28 dynes/cm.^{2} and in energy density measurement = 0·28 Ergs/cm.^{3}. In this measurement, contribution due to streaming was avoided. Only pressure due to radiation was taken into account.

5. DETERMINATION OF THE MAXIMUM VELOCITY OF STREAMING THROUGH THE ANNULAR SPACE

There are always some particles in suspension in liquids. These are not ordinarily visible to naked eye but become visible when illuminated by means of some light source For determining the streaming velocity, the movements of these particles are taken into consideration.

In the present investigation, two marks were put on the outer glass tube. The distance between the two marks was 10 cm. Sufficient margin at the two ends of the inner tube was allowed while putting the marks. These margins were allowed to avoid the end effect of the inner tube. The light source was placed in such a position and focused in such a way that the particles were made visible distinctly for the entire journey and at the same time eyes were protected from direct flash of light. For effective observation of the particles, it was necessary to train the eyes by observing constantly for some time. The transit time of the particles to cover the known distance of 10 cm. was measured by means of a stop watch. In this case also, recording the transit time correctly was practised several times before taking the final readings. In the measurement of streaming velocity personal factor plays an important rôle. So, in the present case maximum effort was made to minimize the personal factor. Maximum streaming velocity was determined from the minimum transit time. For this, several observations were to be made.

6. DETERMINATION OF ULTRASONIC ABSORPTION COEFFICIENTS

Equation (13) shows how the ultrasonic energy density at some place in the sonic path is associated through the absorption coefficient with ultrasonic energy density at the source. By applying the equation (16), the absorption coefficient was determined from the knowledge of ultrasonic energy density and the maximum streaming velocity. The accuracy of the absorption coefficient depends upon the accuracy of the determinations of sonic energy density and the streaming velocity. In the present set of experiments, these two parameters were measured with an accuracy according almost the theoretically expected value and thereby increasing the accuracy of the evaluated values of ultrasonic absorption coefficient.

7. OBSERVATIONS AND RESULTS

Investigated liquids in the present experiment were

(*a*) (i) Water, (ii) ethyl alcohol, and (iii) methyl alcohol;
(*b*) (i) Carbon disulphide, (ii) benzene, and (iii) carbon tetrachloride.

In the case of liquids in group (*b*), which have got abnormally high ultrasonic absorption coefficient, no deflection of the detecting disc was obtained. This means that no force acted upon the disc. This is possible only when whole of the acoustic energy is absorbed within the acoustic path length less than 17 cm. This is a very striking observation. For the detailed investigations of this situation, an experimental arrangement, expecting to befit the situation, is being made.

The results obtained in the present investigations are shown in Table I.

8. DISCUSSION

It is clear from the equation (16) that the streaming velocity is governed by the ratio of the sound absorption coefficient to the viscosity. This shear viscosity is introduced into the theoretical development through the viscous forces resisting steady incompressible flow and is therefore identified with the viscosity coefficient measured, for instance, by the rate of flow through the annular space between two co-axial tubes. Here the second coefficient of viscosity μ', if it influences the streaming at all, will be through its relationship to the coefficient of sound absorption α.

In the present investigation, acoustic energy density was measured from the measurement of the pressure developed on the disc due to radiation only. Streaming effect was cut off by the introduction of the screen. Here these processes were separately treated. But Piercy and Lamb following Nyborg measured the total pressure without differentiating the different mechanisms supposed to be responsible for the dissipation of acoustic energy. The change of radiation pressure within short distance from the source of energy can be effectively measured, if the radiation and the streaming are separated out one from the other. According to Borgnis' (1953) theoretical postulate and the experimental verification of the same by Borgnis himself and Cady and Gittings (1953) the sum of the pressures due to the radiation and the streaming remains constant up to a certain distance (approximately) 25 cm. (experimental figure) from the source. This finding was also

TABLE I

Acoustic energy density, streaming velocity and coefficient of absorption by streaming method

S. No.	Liquids	Energy density at a distance of x cm. from the source E_x-Ergs/cm.^{3} Max. possible error, expressed in per cent	Streaming velocity cm./s		$\alpha/f^2 \times 10^{14}$ db/m. sec.^{2}						
			Average min. transit time to cover 10 cm. t-in secs.	V cm./s	Theoretical	Author's 29°-30°C. f=11 Mc/s	Values from other technique	T°C.	Frequency in Mc/s	Reference (see below)	Remarks
1	2	3	4(a) *	4(b)	5	6	7	8	9	10	11
1	Water	18·12±1·5%	$\left.\begin{matrix}6{\cdot}8\\6{\cdot}9\\7{\cdot}0\\7{\cdot}2\end{matrix}\right\}$ = 7 secs.	1·43	5·5 at 30°C.	7·2	16·6	30°C.	7·5 to 70	P_1	Negative temperature coefficient of absorption
2	Methyl alcohol	14·17±2·0%	$\left.\begin{matrix}3{\cdot}4\\3{\cdot}5\\3{\cdot}6\end{matrix}\right\}$ = 3·5 secs.	2·86	11·4 at 30°C.	13·9	28·2	30°C.	15	PG	do.
3	Ethyl alcohol	12·78±2·2%	$\left.\begin{matrix}2{\cdot}7\\2{\cdot}9\\3{\cdot}0\\2{\cdot}8\end{matrix}\right\}$ = 2·9 secs.	3·45	19·9 at 30°C.	30·7	42·3	30°C.	52·4	P_2	do.
4	Carbon disulphide	Could not be determined				Could not be determined	6,400	23·27		W	No deflection of the light spot was observed
5	Benzene	do.				do.	695	23·5	15	PG	do.
6	Carbon tetra chloride	do.				do.	463	21·8	15	PG	do.

P—Pinkerton, J. M. M. (1) (1947). *Nature* (*Lond.*), **160**, 128.
(2) (1949). *Proc. Phys. Soc. Sec. B* (*Lond.*), **62**, 129.
PG—Pellam, J. R., and Galt, J. K. (1946). *J. Chem. Phys.*, **14**, 608.
W—Willard, G. W. (1941). *J. Acoust. Soc. Amer.*, **12**, 435.

* Time of traverse to cover 10 cm. by particles in different parts.
Water-t sec = 8·0, 8·2, 8·8, **6·8**, 10·0, **6·9**, 7·8, **7·0**, **7·2**, 7·5.
Methyl alcohol-t sec = 4·2, **3·4**, 4·5, 5·0, **3·5**, 3·7, 3·9, 4·3, 4·2, **3·6**.
Ethyl alcohol-t sec = 4·5, 4·2, 3·1, 3·6, **2·7**, **2·9**, **3·0**, 3·5, **2·8**, 3·2.
Average values are taken from the bold figures.

Column 3 shows the experimentally determined values of acoustic energy density; column 4(b) the streaming speed; column 5 shows the theoretical value of $\alpha/f^2 \times 10^{14}$ based on shear viscosity only at the temperature noted against each figure; column 6 shows the author's value of $\alpha/f^2 \times 10^{14}$; column 7 indicates these values as obtained by other authors from other technique; columns 8 and 9 show respectively the temperature and the frequency at which the values of $\alpha/f^2 \times 10^{14}$ were obtained by other authors. Column 10 gives symbolic name of the other authors. Footnote (*) shows the records of time required by particles present in different liquids to cover a distance of 10 cm. From these records the minimum transit time was found out.

found to be approximately true by Bhadra (1958) up to a distance of about 8 cm., and this is due probably to the onset of turbulence. Therefore, by measuring the total pressure, the difference of pressure between the ends of the tube cannot be ascertained. The direction of streaming through the return path which is outside the main beam is opposite to that of the acoustic beam.

This phenomenon can be well appreciated, if the Borgnis' idea that streaming pressure increases exponentially and radiation pressure decreases exponentially with distance is incorporated here. Naturally, streaming pressure at the far end from which the flow starts outside the main beam is greater than that at the end nearer the source of energy, and therefore the flow is in the opposite direction. For clear understanding of the above process it seems reasonable to consider streaming and radiation separately.

Summarized results obtained in the present investigation are shown in Table I. For the three substances in group (*a*), acoustic energy density, and streaming velocity, were determined experimentally. But in the case of the three substances in group (*b*), which are highly absorbing, these two quantities could not be measured as the indicating system did not show any change. This means that no energy reached the detecting disc.

The values of α/f^2 as determined by employing the equation (16) from the measured $\dot{E}_x$ and $v(r)$ max are shown in column 6. For a comparative study, the theoretical values as calculated from the normal viscosity and the values as obtained from other techniques by other authors and the conditions of their experiments are shown in columns 5, 7, 8 and 9. Comparing the results shown in columns 5, 6 and 7 it is found that the results obtained by the author approach nearer to the theoretical values compared to those obtained by other authors.

From the findings of the present investigations, it may be inferred here that the measurements of the absorption coefficient is very much sensitive to the experimental conditions and arrangements. If the experimental arrangement be such that the surface area of the effective part of the apparatus is large in comparison with the volume of the liquid flowing through, the normal viscosity plays a dominant part. Richardson (1954) in his capillary tube experiment attributed the cause of absorption to the skin friction and showed that the first viscosity was adequate to predict the attenuation. The cause for the lower values of the absorption coefficient as obtained in the present investigation may be attributed to be due to skin friction effect, which involves shear type of motion. As to the reason for the variation of α_{obs} as obtained in the preceding investigation (Cady and Gittings' method) and in this investigation (Piercy and Lamb's modified method), it is suggested that in the former method a part of the energy of streaming is converted into vortices, and therefore the conservation of the momenta of these two is not maintained, while in the present investigation, since the cross-section of the tube enclosing the transducer is a little smaller than the former, there is no opportunity for vortices being formed.

In the case of water, methyl alcohol and ethyl alcohol, the ratio of $\alpha_{obs}/\alpha_{cal}$ comes out to be 1·31, 1·22 and 1·54 (approximately) respectively as against 3·1, 2·47 and 2·12 respectively as obtained by Pinkerton and Galt and Pellam.

Further, from the results shown in columns 4*b* and 6 of Table I, it is apparent that streaming velocity is proportional to absorption coefficient.

But in the case of the three highly absorbing liquids, carbon disulphide, carbon tetrachloride and benzene, no indication of the presence of acoustic energy was observed at a distance of about 17 cm. from the quartz crystal transducer. This means that whole of the acoustic energy is dissipated by the medium within a distance less than 17 cm.

from the source. No streaming of the liquid was observed and also the thermometer (held at a distance of about 19 cm.) did not show any rise of temperature. Further, it has been observed that there was a little local agitation in the liquid just in front of the quartz crystal. From these observations it may be supposed that the input ultrasonic energy is not absorbed as internal energy (vibrational, rotational and translational), ultimately dissipating into heat but is expended in other processes such as generation of turbulent motion in front of the quartz crystal.

The experiments and theories of these, at least under the conditions investigated, indicate that radiation force may be a more complicated phenomenon associated with acoustic wave propagation than has hitherto been supposed.

For elucidation of these points, modified experimental arrangements are being made.

9. ACKNOWLEDGEMENT

The author wishes to express his grateful appreciation to Dr. D. M. Bose, Director, Bose Institute, Calcutta, for his keen interest, constant encouragement and critical discussions during the progress of the work.

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XII. INDUCING MUTANTS WITH ECONOMIC CHARACTERS IN TIL (*SESAMUM*) AND RAPE AND MUSTARD (*BRASSICA*) BY X-RAYS

By U. K. Rai *and* K. T. Jacob

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INTRODUCTION

The usefulness of X-rays in inducing chromosome aberrations and genetic variability came to be recognized since the publication of the results of classical experiments in *Drosophila* by Muller in 1927. But some of the subsequent investigators in this line, like Stadler (1928 and 1930) and others, thought that many of the mutations thus induced were deleterious and hence this technique did not offer much as a tool in plant breeding. But the practical utility of this method in plant breeding was first demonstrated by Gustafsson and his collaborators in 1935 and, since then, they have obtained vital and other mutants with increased yield, disease resistance and other favourable agricultural characteristics in barley, wheat, oats, flax, soybeans, sweet lupines, oliferous species of Cruciferae, etc. In U.S.A. also, this method is being successfully used at present in crop plants such as ground-nuts, lupines, flax, wheat, barley, oats, etc., in which higher yielding and disease-resistant strains have been obtained in a comparatively short period.

Induced mutation experiments have been undertaken in jute at the Bose Institute since 1941; at present, systematic work is being conducted in jute, oil-seeds like sesamum and mustard, cotton, paddy and tomato and interesting results, both from the fundamental and applied aspects, have been obtained. The present paper reviews the important results obtained in sesamum and mustard since the inception of the experiment in 1950.

OBJECTIVE

The aim of the present investigation is to induce desirable hereditary changes in the seeds of til (*Sesamum*) and rape and mustard (*Brassica*) by subjecting them to different doses of ionizing radiations of X-rays. It is known that the hereditary qualities of plants and animal cells reside in the chromosomes, which differ in size, shape and number in different varieties, species or genera. The hereditary qualities can be altered by modifying the position and structure of the gene molecules (nucleoproteins) in the chromosomes. The resulting modifications of the species, called mutations, occur at a very slow rate even under natural conditions. Several physical and chemical agents can be used for accelerating the rate of mutations and one of the most convenient of them is to subject the seeds to different doses of X-rays.

MATERIAL

For this purpose and as required for such studies, stable types of *Sesamum* (Kharif crop) and rape and mustard (Rabi crop) were obtained from the Agricultural Departments of the various States in the Indian Union. The types selected for the present experiments both in *Sesamum* and Rape and Mustard in the first year are listed below, together with their sources.

Sesamum

	Botanical name	Type No.	Somatic chromosome No.	Locality and State
1.	*Sesamum orientale*	Type 10	26	Berhampore, W. Bengal
2.	,, ,,	Type 12	26	,, ,,
3.	,, ,,	Type 16	26	,, ,,
4.	,, ,,	Type 20	26	,, ,,
5.	,, ,,	T.M.V. 1	26	Tindivanam, Madras
6.	,, ,,	T.M.V. 2	26	,, ,,
7.	,, ,,	T.M.V. 3	26	,, ,,
8.	,, ,,	T. 85	26	Nasik, Bombay
9.	,, ,,	N. 41	26	Nagpur, ,,
10.	,, ,,	N. 128	26	,, ,,
11.	,, ,,	T. 10	26	Kanpur, U.P.
12.	,, ,,	N.P. 3	26	Pusa, Bihar
13.	,, ,,	N.P. 7	26	,, ,,
14.	,, ,,	N.P. 29	26	,, ,,

Mustard

	Botanical name	Variety or strains	Somatic chromosome No.	Locality and State
1.	*Brassica juncea*	Rai 5	36	Berhampore, W. Bengal
2.	,, *campestris*	Tori 7	20	Malda, W. Bengal
3.	,, *juncea*	R.L. 9	36	E. Punjab
4.	,, *campestris*	Brown Sarson	20	,,
5.	,, *juncea*	R.L. 18	36	,,
6.	,, ,,	R.L. 19	36	,,
7.	,, *campestris*	Maghi Lahi	20	Kanpur, U.P.
8.	,, ,,	R. type 11	20	,, ,,
9.	,, ,,	Mustard 36	20	Sabour, Bihar
10.	,, ,,	Mustard 40	20	,, ,,
11.	,, ,,	Mustard 325	20	,, ,,
12.	,, *juncea*	T. 164	36	I.A.R.I., New Delhi
13.	,, ,,	T. 185	36	,, ,,
14.	,, *campestris*	T. 88	20	,, ,,
15.	,, ,,	T. 71	20	,, ,,

Of these, Nos. 1 to 6 of *Sesamum* and Nos. 1 to 4 of Mustard thrived well under the local conditions and hence were selected for further detailed investigations. In 1953, two strains of *B. juncea*, viz. T. 164 and T. 185, and two of *B. campestris*, viz. T. 88 and T. 71, received from the I.A.R.I., New Delhi, were also included in these experiments.

SUMMARY OF THE WORK DONE

In the course of these experiments during the last seven years, interesting results of economic and fundamental importance have been obtained, both in *Sesamum* and Mustard. These are being classified under two main heads, (i) those having economic importance and (ii) those of fundamental importance, and are as follows:—

Economic importance

High yielding mutants selected in the X_1 generation

In the irradiated progenies, increase in the number of fruits and weight of seeds per plant over the control have been obtained in all the types of *Sesamum* and Mustard under investigation. Table I gives the comparative record of the yield of fruits of the selections in the treated as well as control progenies in various generations studied during the period. For comparison and proper evaluation of the data, the number of fruits in the best plant both in the controls and treatments have been included in Table I and the number of fruits and the total weight of the seeds in the best plants have been included in Table II, as although the number of fruits produced by a plant gives an indication of its yielding capacity and hence a useful index for purposes of selection in the field, the absolute yield has to be measured in terms of the seeds produced by it.

From the data in Tables I and II, it can be seen that the yields with reference to the total number of fruits and the total weight of the seeds have increased in the progenies of the treatments over their respective controls. In some cases, there were fluctuations in the yield which was probably due to the heterozygosity produced by irradiation, affecting the complex yield behaviour, the probable causes of which are being dealt with subsequently.

TABLE I

Crop	Type and locality	Control and treatment	Number of fruits in the best plant					
			X_1	X_2	X_3	X_4	X_5	X_6
S. orientale	Type 12 (W. Bengal)	Control	150	77	150	241	212	262
		36 mAh	140	178	200	199	252	553
,,	Type 16 (W. Bengal)	Control	121	116	185	168	170	244
		36 mAh	125	164	348	234	560	478
		50 ,,	144	193	302	280	208	356
,,	Type 10 (W. Bengal)	Control	270	238	356	275	..	..
		100 mAh	487	456	316	320	..	..
		140 ,,	325	488	275	310	..	..
,,	T.M.V. 1 (Madras)	Control	205	220	150	240	221	..
		36 mAh	281	212	262	218	312	..
		50 ,,	601	328	261	315	410	..
		100 ,,	243	336	206	400	379	..
Brassica juncea	Rai 5 (W. Bengal)	Control	455	1,250	890	285	..	..
		50 mAh	665	2,300	1,268	548	..	..
		65 ,,	437	490	863	420	..	..
		100 ,,	552	725	1,120	336	..	..
		140 ,,	547	410	1,108	640	..	..
B. campestris	Tori 7 (W. Bengal)	Control	460	312	210	..	..	..
		65 mAh	460	395	287	..	..	..
		100 ,,	380	375	291	..	..	..
		140 ,,	450	456	364	..	..	..
		200 ,,	508	430	321	..	..	..
,,	Brown Sarson (E. Punjab)	Control	394	841	177	..	..	..
		65 mAh	198	300	725	..	..	..
		100 ,,	662	870	267	..	..	..
		200 ,,	321	708	540	..	..	..

TABLE

Crop	Type and locality	Control and treatment	Number of fruits and the weight of seeds in the best plant in the control and treatments of the various generations							
			X_3		X_4		X_5		X_6	
			No. of fruits	Weight of seeds in gms.	No. of fruits	Weight of seeds in gms.	No. of fruits	Weight of seeds in gms.	No. of fruits	Weight of seeds in gms.
Sesamum orientale	Type 12 (W. Bengal)	Control	..	..	..	..	212	34·10	262	13·54
		36 mAh	..	..	..	..	252	34·80	553	28·16
,,	Type 16 (W. Bengal)	Control	..	..	..	..	170	23·60	244	14·40
		36 mAh	..	..	..	..	542	38·34	348	23·61
		50 ,,	..	..	..	..	465	31·04	318	20·62
,,	T.M.V. 2 (Madras)	Control	..	..	79	8·67	110	9·65	..	..
		50 mAh	..	..	227	10·45	300	13·86	..	..
		100 ,,	..	..	224	15·22	375	15·45	..	..
,,	T.M.V. 1 (Madras)	Control	..	..	240	19·64	221	13·11	..	..
		36 mAh	..	..	290	28·86	370	18·96	..	..
		100 ,,	..	..	400	25·39	379	18·76	..	..
,,	Type 10 (W. Bengal)	Control	336	23·83	275	10·46	..	..	..	..
		100 mAh	316	30·68	320	21·76	..	..	..	..
		150 ,,	372	20·70	254	18·62	..	..	..	..
,,	Type 20 (W. Bengal)	Control	368	31·54	275	16·21	..	..	..	..
		100 mAh	208	17·25	212	17·39	..	..	..	..
		140 ,,	304	30·88	331	19·62	..	..	..	..

TABLE III

Crop	Type and locality	Control and treatment	Generation					
			X_1		X_2		X_3	
			Age at first flowering, in days	Earliness in days	Age at first flowering, in days	Earliness in days	Age at first flowering, in days	Earliness in days
Sesamum orientale	Type 16 (W. Bengal)	Control	48		45		46	
		36 mAh	43	5	36	9	36	10
B. juncea	R.L. 9 (E. Punjab)	Control	51		67			
		50 mAh	48	3	60	7		
		65 ,,	45	6	63	4		
		100 ,,	48	3	65	2		

In addition to the high yielding mutants, early maturing mutants have also been isolated and in some cases it has been noted that the higher yielders were also early maturing. Table III gives the behaviour of some of these early mutants in the subsequent generations.

These early flowering progenies have been selected as individual plants in the X_1 generations and from their behaviour up to the X_3 generation appear to retain their character for earliness. But they have to be studied for a few more generations before their practical utility can be assessed.

Quality and quantity of the oils extracted from irradiated progenies and control

The oil was extracted from the mutants as well as other selections of the irradiated progenies with the Soxhlet apparatus and the results from a few are included in Table IV. The colour of the oil, as determined by the Lovibond scale and their keeping qualities as determined by their peroxide value, have been included in Tables V and VI respectively. On analysis, it was noted that there were no changes in the mixed fatty acids, refractive index, sap value, iodine value and percentage of non-sap, etc., between the controls and the treatments.

TABLE IV

Crop	Type and locality	Control and treatment	Percentage of oil in	
			X_1	X_5
Sesamum orientale	Type 12 (W. Bengal)	Control	36·0	41·1
		36 mAh	40·0	56·0
,,	Type 16 (W. Bengal)	Control	39·0	40·77
		36 mAh	45·0	44·04
		50 ,,	38·0	42·80

TABLE V

Crop	Type and locality	Control and treatment	Colour by Lovibond scale
Sesamum orientale	Type 16 (W. Bengal)	Control	0·4 R 2·1 Y
		14,400 r	0·0 R 0·9 Y
		20,000 r	0·1 R 1·1 Y
		40,000 r	0·0 R 0·8 Y
		56,000 r	0·0 R 0·7 Y
Brassica juncea	Rai 5 (W. Bengal)	Control	2·2 R 40·0 Y
		20,000 r	1·2 R 40·0 Y
		26,000 r	1·2 R 42·0 Y
		40,000 r	1·4 R 40·0 Y
		56,000 r	1·0 R 40·0 Y

TABLE VI

Crop	Type and locality	Control and treatment	Peroxide value		
			Freshly extracted oil	Oil kept for some days *	Percentage of increase
Sesamum orientale	Type 12 (W. Bengal)	Control	6·3	15·4	144·4
		56,000 *r*	5·2	12·5	140·3
B. juncea	Rai 5 (W. Bengal)	Control	9·9	27·4	176·8
		20,000 *r*	6·0	11·5	92·6
		26,000 *r*	6·5	12·8	96·9
		40,000 *r*	7·5	16·8	124·0
		56,000 *r*	8·7	20·2	132·2

* Kept for seven days in the case of *Sesamum* and one month in the case of *Brassica.*

As seen from Table IV, the oil content was higher in the irradiated progenies than in the control. For purposes of comparison, the relative oil content in the X_1 and X_5 generations in *Sesamum* types 12 and 16 are given. From Table V, it appears that the red pigment was practically absent from the oil of the irradiated *Sesamum* progenies, while the yellow pigment was reduced to about half. Hence the oil appeared to be clearer. In mustard, the red pigment was reduced to about half, while there was little change in the yellow pigment and here also the oil appeared to be clearer. Again, as seen from Table VI, the peroxide value of the freshly extracted oil was lower in the irradiated progenies than in the control both in *Sesamum* and Mustard, and even when the oil was kept for some time, the increase in the percentage of the peroxide value was lower in the irradiated progenies. This is a very important aspect.

Pollens

Since the size of the pollen grains and the percentage of their sterility are important factors in assessing the behaviour of the plants, these were studied in the irradiated progenies from the X_1 generation onwards, in comparison with the controls. The results in *Sesamum* types 12 and 16 are summarized in Table VII. As seen from this

TABLE VII

Crop	Type and locality	Control and treatment	Pollen			
			X_1		X_7	
			Sterility	Diam.	Sterility	Diam.
S. orientale	Type 16 (W. Bengal)	Control	3·46	65·95	2·06	60·11
		36 mAh	5·17	67·23	2·55	60·42
		50 ,,	5·32	70·17	2·20	60·20
,,	Type 12 (W. Bengal)	Control	4·62	66·12	3·24	57·19
		36 mAh	5·00	71·94	3·45	57·15
		50 ,,	7·04	71·14	3·50	59·71

table, although these differed from the controls in the X_1 generation, in the X_7 generation there were very little differences and hence it can be surmised that the irradiated progenies in the X_7 generation are as stable as the parent plants.

Morphological mutants

Morphological recessive white-flowered and small-seeded mutants have been isolated from *Sesamum orientale* type 16 in the X_8 generation of 20,000 *r* and the X_4 generation of 14,400 *r* treatments respectively. They have been breeding true for three to four generations as regards their yield of fruits, seeds and percentage of oil.

In these mutants, although there were no visible differences in the plant heights, branching and flowering behaviour in relation to the controls, there were differences in the oil content, as seen from Table VIII, where the small-seeded mutant has a significantly higher oil content of about 25 per cent and the white-flowered mutant about 6 per cent over the control. These are being multiplied for large-scale trials.

TABLE VIII

Mutant and control	Mean percentage ±S.E.	Mean percentage over control ± S.E.
Control (type 16)	41·49 ± 0·51	100·00 ± 1·23
Small-seeded	52·10 ± 0·51	125·56 ± 1·23
White-flowered	44·06 ± 0·51	106·18 ± 1·23

A white-flowered mutant isolated in *Brassica juncea*, type Rai 5, in the X_2 generation of the 26,000 *r* treatment, is breeding true for the flower colour, which in the X_3 generation also gave a greater number of fruits over the control.

CYTOLOGICAL STUDIES

Somatic chromosomes

All the *Sesamum orientale* types under investigation have $2n = 26$ chromosomes. Mustard types Rai 5, R.L. 9, T 185 and T 164, belonging to *Brassica juncea*, an amphidiploid, have $2n = 36$, whereas types Tori 7, Brown Sarson, T 71 and T 88, belonging to *Brassica campestris*, have $2n = 20$ chromosomes. The morphology of the chromosomes and changes brought about, if any, due to irradiation could not be clearly made out, due to the difficulties experienced in fixing and staining the complement, which is composed of relatively small chromosomes. However, irradiations have not changed the total number of chromosomes in the species studied.

Irradiation induced meiotic irregularities

The study of the type of the chromosomal aberrations encountered during the microsporogenesis helps in evaluating the complex disorder which may be brought about in the gametes and thereby change the genic pattern of the chromosomes.

Sesamum orientale

The nucleolus show a tendency to persist at metaphase and anaphase and sometimes these have been observed in the diad and tetrad stages also. Persisting nucleolus have sometimes been found to occur with the clumping of the chromosomes. This clumping may involve all the 13 bivalents in a group or give rise to a chain-like appearance; the rest of the bivalents, however, form a separate micronucleus. Non-orientation, clumping and scattering of univalents due to the disorganization of the spindle mechanism, result in irregularities at subsequent stages. The lagging of maximum of three bivalents and extrusion of maximum of two bivalents at Telophase I show the gross changes brought about in these. Clumping, extrusion and formation of micronucleus and tripolar spindle are some of the common irregularities at the second division also.

Brassica juncea

Clumping and false association (four or five associations) are the common irregularities at the prophase and metaphase stages. A maximum of two univalents has been observed at Metaphase I, which in the anaphase stage passes to the same pole in a highly disturbed condition earlier than the bivalents and has been found to form a separate micronucleus in the later stages. Some cells have been observed with eight bivalents and five tetravalents. Inversion bridges with one or two fragments have been observed at the late Anaphase I and Telophase I. Rare cases of cytomixis have also been encountered.

Brassica campestris

Clumping, non-orientation, congregation, scattering and false association of the chromosomes have been often encountered at metaphase stage. A maximum of four univalents scattered near a pole at anaphase has often been noted, out of which two univalents usually form a micronucleus. These, however, appear to have influenced the formation of a separate cell at the tetrad stage. Inversion bridges, together with univalents in the same cell, are important abnormalities in explaining the heterozygosity.

Genetical aspects

The following are some of the important genetical aspects which have emerged from the present study:

(i) The nature and fluctuations in the yield of these crops after various dosages of X-rays.

(ii) The behaviour of a multicoloured-seed mutant of *Sesamum orientale* type 12, isolated in X_4 generation from 56,000 *r* treatment.

1. On plotting the graph for fruit yield of the selections in the various generations after the irradiation, it has been found that (*a*) some of the selections which gave a greater yield than the control in the first generation continued to give increased yield in the subsequent generations, (*b*) other selections which gave a higher yield in the first two generations may at the next generation give a lesser yield when compared to the control and again in the later generation regain its capacity for giving higher yield, (*c*) still others which gave a lower yield than the control in the first generation gave higher yields in the subsequent generations, and (*d*) a few of the selections which gave a lower yield than the control in the first generation continue to give low yield in the subsequent generations.

The yield is a quantitative character and is supposed to be governed by the multiple factors. From the above observations, it appears that the categories '*a*' and '*d*' show homostatic nature whereas '*b*' and '*c*' show heterostatic nature. It cannot be definitely said whether this heterozygosity is at the allelic or genic level. The cytological observations show the presence of inversion bridges as the result of irradiation, which are usually eliminated in subsequent generations. Therefore, this heterozygosity appears to be due to the inversion of a block of genes or a part of chromosomes producing heterozygosity in the alleles or genes respectively. Now if these inversions do not affect the localization of chiasmata, the heterozygosity produced will have the tendency to stabilize in subsequent generations and thus result in the '*a*' or '*d*' type of behaviour. On the contrary, if these inversions affect the localization of chiasmata at one period or the other, '*b*' and '*c*' types of behaviour will result. This is being further studied.

2. *Multicoloured-seed mutants* (seeds with black, brown, grey, ash and white colour) have been isolated from *Sesamum orientale* type 12 which had originally black seeds. The data are being analysed, but it appears that (*a*) three pairs of factors are involved in such a behaviour, one of the pairs being lethal in dominant homozygous state, and (*b*) two different parallel lines of the development of colour also take place, based on simple Mendelian ratio.

Thus these observations are direct evidences in establishing the usefulness of ionizing radiations not only from the economical aspect, but also from the theoretical point of view, in understanding the genic constitution of plant and thus helping in preparing the linkage maps.

The preliminary information regarding study of the Mendelian inheritances of hairiness of the capsules and the morphology of the seeds both in *Sesamum* and Mustard have already been obtained and further studies are being conducted.

Morphological

The marked difference in the root system, shape, thickness and colour of leaf, pigmentation in the plant parts, phylloidy, etc., are some of the useful observations recorded during the period.

ACKNOWLEDGEMENTS

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XIII. CYTOLOGICAL RESPONSES OF PLANTS TO AMINOTRIAZOLE

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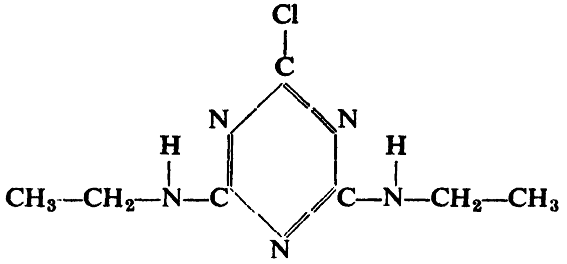
Introduction

Aminotriazole—a grey organic compound with the following constitution—is one of the more recent weedicides and hence many of its properties and action remain yet to be tested.



Besides being a herbicide this can also be used as defoliant in cotton and such other crops. In course of preliminary weed control experiments with this chemical, peculiar growth inhibiting properties following the treatment were noticed. It therefore appeared interesting to test its cytological effects on plants.

A sodium salt of Aminotriazole, which is soluble in water and is known to be almost non-toxic to the animals, was used in the present investigations.

Materials and method

The tests were conducted by treating 4-day old seedlings of *Pisum sativum* L. grown from seeds obtained from Sutton's Nursery, as a stable strain under the varietal name 'Duke of Albany'.

Aminotriazole was used in four concentrations, viz. 100, 200, 1,000 and 5,000 p.p.m. (active ingredient). Twelve seedlings were immersed in each one of the concentrations, for a duration of 2 and 24 hours respectively. Two-thirds of the seedlings were revived in running water for the durations specified below. Following the chemical treatments, the seedlings were divided into three lots of four seedlings each as follows:—

(*a*) Washed and fixed immediately after.

(*b*) Recovered in running water for 24 hours and fixed.

(*c*) Recovered in running water for 48 hours and fixed.

Corresponding controls were similarly kept in distilled water and fixed along with treated seedlings.

The root tips were fixed in a mixture of rectified spirit and HCl (conc.) in the ratio of 3 : 1. They were transferred to aceto-carmine stain after 1½ hours and squashes were made following usual iron aceto-carmine technique, which gave satisfactory and quick results.

Some root tips from each treatment were fixed in acetic alcohol (1: 3) and stained in leuco-basic fuchsin, for testing the effect of these treatments on the stainability of the chromosomes.

OBSERVATIONS

Effect of the chemical on the chromatin.—The leuco-basic fuchsin stainability of the chromosomes was taken as the index of the action of sodium-aminotriazole on the chromatin. It has been found that up to a concentration of 1,000 p.p.m. and for the 2 and 24 hours' durations of the treatment, the chemical has no marked effect on the nucleic acid content of the chromosomes in that the chromosomes take almost as bright Feulgen's stain as in the control.

The organization and behaviour of nucleolus also do not show any differences from the control.

At 5,000 p.p.m., as the cell division comes to a standstill, most of the nuclei were found to be in the resting stage and hence the nucleic acid response as indicated by the stainability could not be ascertained.

Effect of the treatment on the cell division.—The number of dividing cells in each of the treatments was taken on the average of the counts from treated root tips. It was found that the largest number of the dividing cells were present in case of 200 p.p.m. treatment while the lowest was met with in 5,000 p.p.m. concentration of the chemical. The frequency of division in other treatments and in the control ranged between these.

The data on the number of dividing cells per root tip in each treatment is included in Table I.

TABLE I

Effect of aminotriazole on the mitosis of Pisum *sp.*

Concentration of the chemical in p.p.m.	Duration of treatment in hours	Revival in hours	Total No. of dividing cells	Stage of cell division			Percentage of anaphasic ab-normality
				Metaphase	Anaphase	Telophase	
Control	..	..	175	87	78	..	1%
		24	170	80	85	5	,,
		48	140	65	76	4	
100	2	..	140	65	69(8)	6	11·7%
	,,	24	138	87	49(5)	2	10·2%
	,,	48	126	84	41(6)	1	14·5%
	24	..	89	73	12(1)	4	8·3%
	,,	24	68	55	10(3)	3	30·0%
	,,	48	57	38	19(3)	0	15·8%
200	2	..	195	161	32(5)	2	15·6%
	,,	24	190	120	69(10)	1	14·5%
	,,	48	185	112	73(20)	0	27·1%
	24	..	34	30	4(1)	0	25·0%
	,,	24	36	29	5(2)	2	40·0%
	,,	48	36	30	6(2)	0	33·0%
1,000	2	..	123	82	40(10)	1	25·0%
	,,	24	109	95	14(4)	0	28·5%
	24	..	32	28	4(3)	0	75·0%
	,,	24	21	19	2(1)	0	50·0%
5,000	2	..	24	16	8	0	..
	,,	24	19	12	7	0	..
	24	..	6	5	1	0	..
	,,	24	9	8	1	0	..

( ) Figures in brackets represent the number of aberrant divisions.

I. *Control:*

The distribution of metaphase and anaphase stages in the total number of dividing cells is roughly equal. The anaphasic abnormalities are very few and only a single case of laggards was recorded.

II. *Treatments:*

A. *Frequency of divisions*

Fixing the root tips immediately after treatment.—Two durations of treatment (2 and 24 hours) were given in all the four concentrations, i.e. 100, 200, 1,000 and 5,000 p.p.m., and the root tips were fixed immediately after the treatment. The following observations are made:—

The number of divisions per root is highest in control. It remains almost unaffected at the 100 p.p.m. while the 200 p.p.m. treatment brings about an appreciable increase in the frequency of division. The 1,000 and 5,000 p.p.m. treatments, on the other hand, bring about a lowering in the frequency. This lowering, though not proportional to the concentration, is directly related to the duration of treatment. Thus due to a 5,000 p.p.m. treatment for 24 hours, the total number of dividing cells per root tip is reduced to 24.

B. *Recovery tests*

(i) *Fixing the root tips after keeping them in running water for 24 hours:*

There is apparently no recovery of the seedlings as indicated by its mitotic activity, by keeping them in running water. There is a proportionately greater loss in the frequency of the divisions in the treatments than in the control. However, the concentration and the duration of exposure have a corresponding effect on the frequency of divisions even after 24 hours in water.

(ii) *Fixing after 48 hours in running water:*

There is a gradual reduction in the frequency of cell divisions per root tip and there is no recovery of the seedlings even after keeping them in running water for 48 hours. The concentration and the duration of treatment still have their corresponding effect.

C. *Relation between the frequency of metaphase and anaphase*

The number of metaphase and anaphase stages are roughly equal in the control, whether grown on moist filter paper or in running water, for the different durations. But as a result of the chemical treatment, a gross reduction in the frequency of anaphase over metaphase is brought about. There is an indirect relationship between the concentration and duration of the treatment on the one hand and the frequency of anaphase stage on the other, i.e. there is a decrease in the number of the anaphase with the increase of the concentration of treatment. The implications of this phenomenon will be discussed later.

D. *Nature and the frequency of chromosomal abnormalities*

The nature as well as the frequency of the chromosomal abnormalities varied from treatment to treatment (Table I). It was observed that despite a very high number of dividing cells in the controls, the frequency of mitotic aberrations does not exceed 1%. In case of the treatments, however, the abnormalities may be summarized as follows:—

(i) *100 p.p.m.:*

The percentage of the aberrations, in the seedlings treated with 100 p.p.m. aminotriazole, ranges between 8·3 and 15·8. There is apparently no definite relation with the time of treatment as well as the duration of washing in running water.

(ii) *200 p.p.m.:*

At 200 p.p.m. treatment, however, there is a definite increase in the frequency of aberration over the 100 p.p.m., being between 15·6 and 40·0%. This treatment also has a corresponding increase in aberrations due to the longer duration of treatment. But if the seedlings are kept in running water, there is no definite change in the frequency of aberrations.

(iii) *1,000 p.p.m.:*

The frequency of aberrations ranged between 25 and 75%. There is a definite increase in the frequency of aberrations with the increased duration of treatment. But the high figure of 75% may also be partly due to a low number of dividing cells. Keeping in water again did not seem to produce any definite effect.

(iv) *5,000 p.p.m.:*

The number of dividing cells was extremely low (ref. Table I). Hence the percentage of aberrations was not reckoned in this case.

The main types of aberrations were late separation, stickiness, condensation of chromosomes, polysomaty, bridges, laggards, extrusion (non-congression) and tripolar spindles, etc. In the higher doses, however, the main abnormality was the reduction in the frequency and delay in mitosis till at 5,000 p.p.m. there is almost a complete inhibition of mitosis. The nature and the consequences of these abnormalities will be discussed later.

DISCUSSION

Since the pioneering works of Levan (1939) on the action of colchicine on the 'root-swelling' of *Allium*, a very large number of chemicals particularly those of phenol and phenoxy acetic groups have been thoroughly investigated for their mutagenic and radiomimetic properties. With the increasing consciousness of the cytological responses of plants to chemical treatments, attention was naturally diverted to find an interpretation of different growth phenomena following treatments with the hormonal herbicides in the behaviour of cell. In this connection the works of Reyland (1948), Nygren (1949), Doxey and Rhodes (1949) and Croker (1953) with 2, 4-D and its derivatives are particularly important in that they have not only reported a large number of cytological abnormalities, but have also shown that in certain concentrations, these chemicals act as inhibitors of cell divisions. The latter, therefore, gives the clue for at least one of the reasons of temporary or the permanent stoppage of growth.

The present investigations with aminotriazole were conducted with almost similar aims to see how far the cytological responses of plants to the various treatments could be held responsible for the phytotoxic action of the substance.

From Table I, it may be seen that the effect of this chemical is twofold:

(i) on the frequency of cell division,

(ii) on the frequency of chromosomal aberrations.

Besides, there is a definite relation between the dose and duration of the treatments.

Effect of aminotriazole on the frequency of cell division.—On analysing Table I with the help of Graphs I and II it may be seen that the 100 p.p.m. concentration does not affect the frequency of cell division to any appreciable extent. In 200 p.p.m. treatment, when exposed for only 2 hours, there is an appreciable increase in the frequency of divisions, suggesting that the quantity of chemical absorbed during the time is sufficient to promote the frequency of division. When exposed to the same concentration for a longer duration or when treated with the higher concentrations of this chemical, it reduces the frequency of divisions, which is roughly proportional to the concentration of the chemical used.

Thus it will be seen that this ratio is maintained in Graph I in the curves *a*, *b* and *c* which further suggests that the passage of time does not improve either the frequency of cell divisions or of aberrations.

Similarly taking Graph II into consideration, where the curves represent the frequency of cell divisions following 24 hours of treatment, it will be seen that the longer duration of the treatment affects the cell divisions more adversely. In other words, the graph not only suggests a low frequency of divisions, but its flat nature suggests an almost complete inhibition of cell divisions, which may be seen in the condition following treatment with 5,000 p.p.m. of the chemical.

Such effects of the chemicals on the plants resulting in the suppression or total inhibition of cell divisions has been recorded previously (Croker, 1953, with 2, 4-D and 2, 4-5T; Greulach and Atchison, 1953, with maleic hydrazide; Doxey and Rhodes, 1949, with M.C.P.A.; Scott and Stuckmeyer, 1956, with C.I.P.C.). Similar are also the responses of some of the contact poisons like chlorates, arsenites and arsenates, but in these the toxicity is the same for all doses and at no concentration the growth promotion is known to occur. Such chemicals affecting the cell divisions have been termed as the 'mitotic poisons' or 'mitostatic substances' (Meletti, 1955). Doxey and Rhodes (1949) and Croker (1953) have found that in treatments when the divisions are not stopped altogether, there is a high frequency of chromosomal aberrations like stickiness, etc. Besides, there is an excessive longitudinal contraction of the chromosomes, which has also been observed in the present experiment. It has been suggested by the authors that the stickiness and excessive contraction of the chromosomes leads to the stoppage of the mitotic activity.

Croker (*loc. cit.*) also noted that there was a direct relationship between the dose of the chemicals applied and the frequency of chromosomal aberrations caused, thereby supporting the 'Chemical Target Theory' put forward by Thoday (1950). In the present set of investigations, this dose-frequency relationship was not absolute, and that at 200 p.p.m. concentration, the frequency of division was enhanced and lowered in all other concentrations. The present observations, therefore, do not conform with the Target Theory in so far as the frequency of division is concerned.

Frequency of cytological aberrations caused by the treatments.—A critical examination of Table I reveals that the dose and duration of treatment have some relationship with the percentage of cytological aberrations. The 'control' has been stated earlier to show a very low frequency of aberrations. But as high as 75% aberrations have been recorded in case of 1,000 p.p.m. treatment. This high percentage of aberrations might be partly due to extreme paucity of divisions.

Effect of recovery in running water.—Recovery of the treated seedlings in running water for a period of 24 to 48 hours has revealed that it does not affect the frequency of chromosomal aberrations to any appreciable extent.

The frequency of cell divisions is, however, influenced to a certain degree by revival. It will be seen from Table I that the duration of revival was also of significance in this connection. In other words, the longer the duration, the more frequent the divisions, except for very high concentrations. In these cases, on the other hand, the concentration of the chemicals has brought about irreversible effects on the seedlings. Such irreversible injuries to the plants both by physical and chemical agencies have been recorded by a large number of other workers also.

Delayed mitosis.—Yet another interesting observation comes from the frequency of metaphases and anaphases. It will be seen that in the control as well as in certain treatments followed by revival, there is a greater similarity in the frequency of the two stages. This suggests of a normal state of division. In the treatments, where no revival of the seedlings was conducted in running water, there is a great disparity between the frequency of the two stages except in case of 100 p.p.m. treatment. In other words, there is a preponderance of metaphase over anaphase which suggests of a delayed process of mitosis or a delayed start thereof. It might, therefore, be concluded that a treatment of over 200 p.p.m. delays the root mitosis in *Pisum* and that a revival in running water for 24 hours or more tends to bring the phenomenon back to the normal. Such delay in mitosis following chemical treatments have been reported among others by Currier (1949) and Doxey and Rhodes (*loc. cit.*).

The reasons for such delays in mitosis are not clearly understood. It appears that not only there is an alteration in the physiological condition of the cell, but also that the properties of cytoplasm are affected to some extent.

Nature of the cytological abnormalities.—The cytological aberrations following this treatment were studied at anaphase only, because in the absence of fragmentation, most of the other abnormalities expressed themselves at the anaphase stage. The observations included laggards, bridges, extrusions, stickiness of the chromosomes, late separation and tripolar spindles.

(i) *Stickiness of the chromosomes:*

Stickiness of the chromosomes is a pathological condition apparently brought about as a result of the chemical treatments. The chromosomes in such cases are clumped together in compact masses in two or three groups (Fig. 1).

But sometimes the chromosomes might be sticky at the ends only, resulting in an obstruction to the normal anaphasic separation. Such sticky chromosomes arising both out of physical and chemical treatments have been reported by other investigators.

(ii) *Lagging chromosomes and late separation:*

The chromosomes lagging on the spindle were frequent (Figs. 2 and 3). These seem to arise perhaps due to insufficient pull of the spindle.

(iii) *Spindle abnormalities:*

No complete failure of the spindle following treatment with aminotriazole could be recorded, as has been seen following treatment with colchicine, phenolic compounds, etc.

In one case a tripolar spindle was observed (Fig. 4) but being a single case, it could not be possible to assess whether it arises due to chemical treatment. However, tripolar and multipolar spindles have been recorded due to chemicals in a number of plants and recently Barthelmess (1957) has made an extensive study of chemically induced multipolar mitosis.

(iv) *Bridges:*

Figs. 5, 6 and 7 represent the anaphasic bridges met with during the present investigations. Such bridges are generally of two types:—

(*a*) Chromatin bridge,
(*b*) Chromosome bridge.

(v) *Condensation of the chromosomes:*

Longitudinal condensation of the chromosomes was very pronounced particularly in the higher concentrations. But the causes for such a reaction could not be traced.

Considering all these factors together, it appears that aminotriazole acts as a 'mitostatic substance' or 'mitotic poison'. When applied to the soil, this substance is assimilated and translocated through the roots so as to affect the meristematic region of both root and the shoot. Depending on the concentration, the cell divisions are either stopped completely or the process is delayed considerably besides the induction of a large number of chromosomal abnormalities which in turn affect the cell division in the subsequent generations, thereby depressing the growth of the plant. In higher doses, the chemical acts as a phytotoxic substance and kills monocots and dicots alike.

However, unlike the weedicides of the 2, 4-D group, it does not appear to be a 'spindle poison'.

SUMMARY

1. The effect of aminotriazole on the root tips of *Pisum sativum* L. has been investigated.

2. The effect of the treatment on the nucleic acid and nucleus of cells has been studied by the leucobasic fuchsin reaction. It has been found that the nucleic acid is not affected even at 1,000 p.p.m. concentration.

3. The chemical has been found to reduce the frequency of cell divisions which in very high concentration acts as a mitostatic substance.

4. The treatment has been found to delay the phenomenon of mitosis in lower doses, as a result of which the frequency of anaphases is reduced.

5. A large number of cytological abnormalities like bridges, sticky chromosomes, excessive longitudinal condensation of chromosomes and late separation are caused following these treatments which have been discussed and interpreted.

6. It has been found that recovery of the treated seedlings in running water for 24 and 48 hours has very little effect on either the frequency of divisions or aberrations.

7. Finally, it has been concluded that one of the reasons for its weedicidal properties is its mitostatic nature of action, which paralyses vital functions in the plant when applied in herbicidal concentration.

ACKNOWLEDGEMENTS

I have the pleasure to acknowledge with gratitude the unfailing help and inspiring suggestions of Dr. K. T. Jacob during the progress of this work.

Thanks are due to Dr. D. M. Bose, Director, for the laboratory facilities and interest in the work and also to Messrs. Agromore Company, Bangalore, for the supply of free samples of aminotriazole under the trade name 'Weedazole'.

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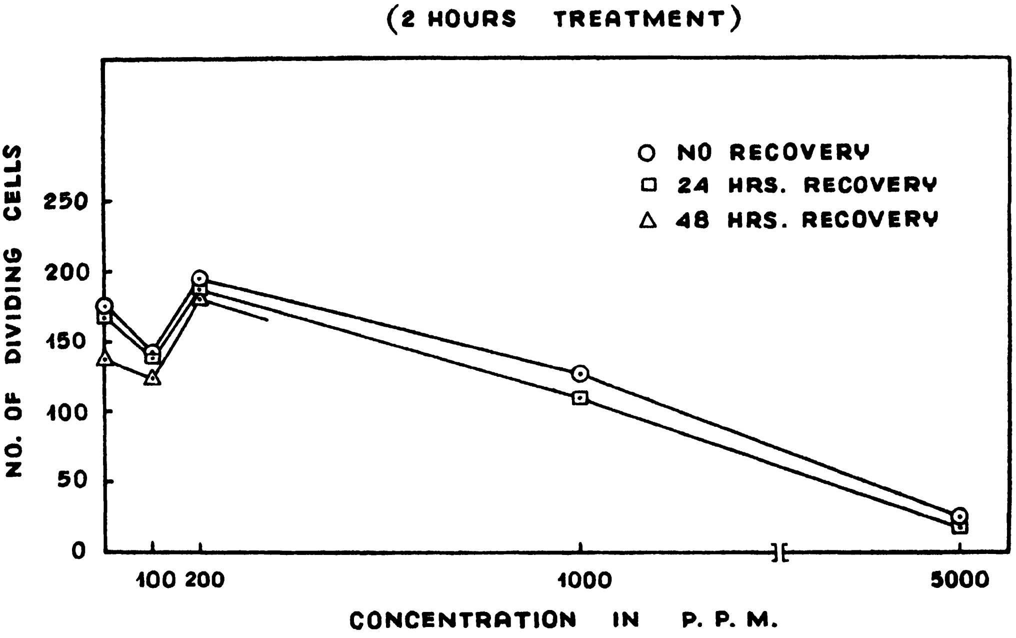
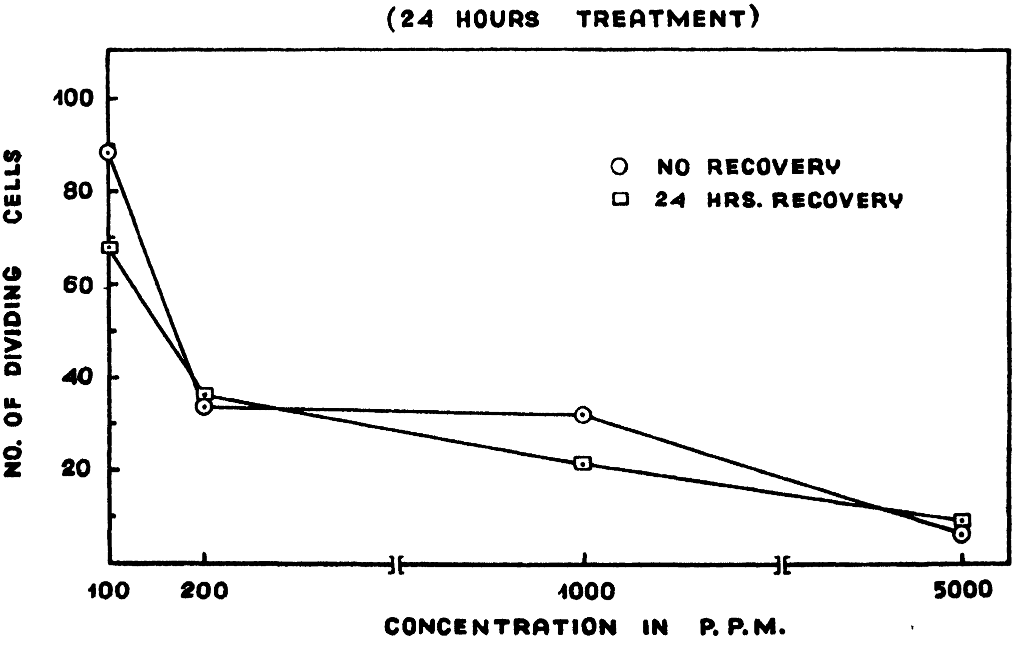
EXPLANATION OF THE FIGURES

FIGS. 1–7 are camera lucida drawings made from a Leitz Ortholux microscope with a 95: 1 objective and 10× compensating eye-piece, giving a table magnification of 2000×. All the figures have been reduced to 2/3 size in the plate. The following is the myth of the figures:—

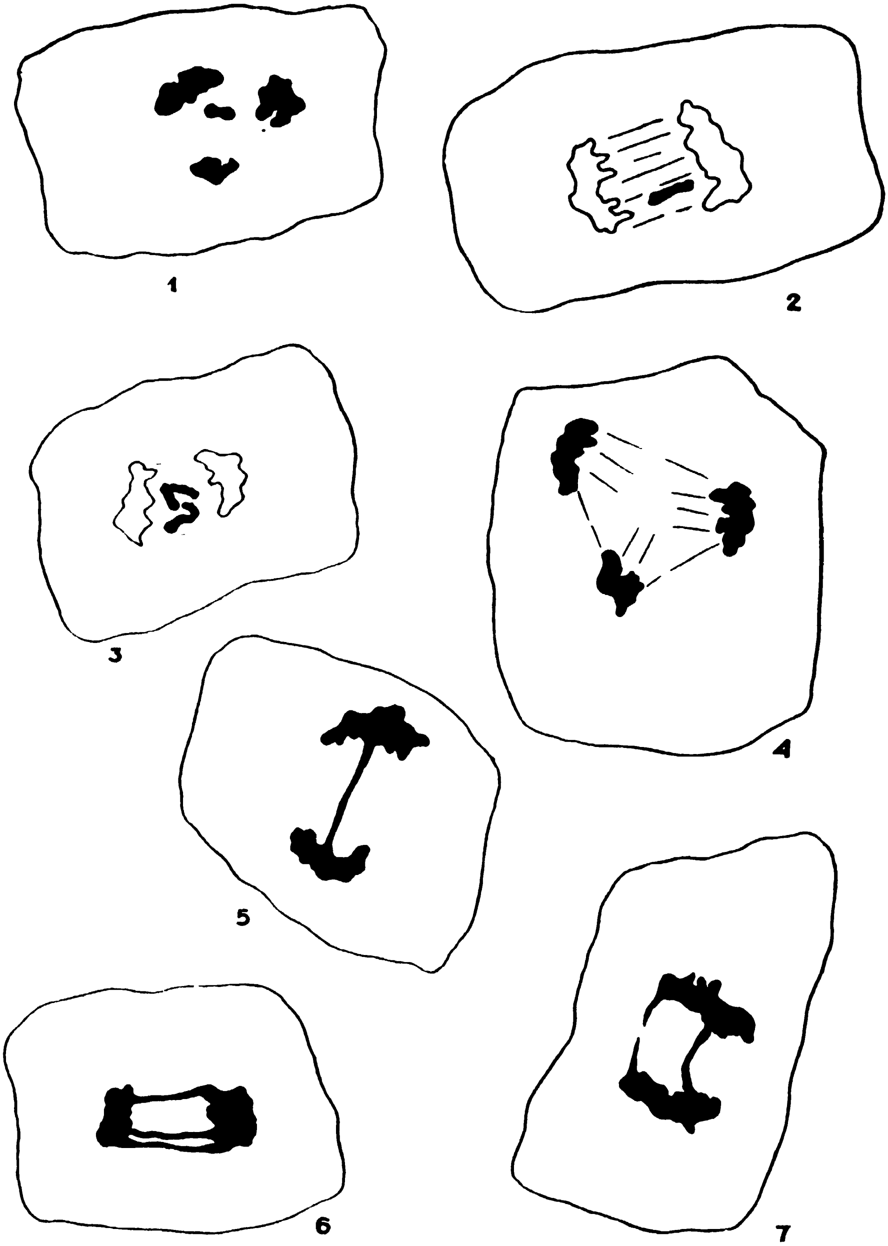
FIG. 1. Metaphase showing three clumps of sticky chromosomes.
,, 2. Anaphase showing one laggard.
,, 3. ,, ,, three laggards.
,, 4. ,, ,, a tripolar spindle.
,, 5. ,, ,, a single bridge.
,, 6. ,, ,, three bridges.
,, 7. ,, ,, a broken and complete bridge.



GRAPH I



GRAPH II



XIV. AUTOGENIC RESPONSIVE MOVEMENT OF THE LEAF OF *MIMOSA PUDICA*

By A. GUHA THAKURTA *and* B. K. DUTT

(*Received for publication on April 22, 1958*)

In the study of the movements of plants the responsive phenomenon of the Mimosa leaf has been most extensively investigated. It is well known how the leaf exhibits its characteristic movement in response to an applied stimulus. The excitatory response of the leaf also depends upon its tonic condition. On a dry sunny day the leaf is in a highly excitable condition, responding to a very feeble stimulus. On the other hand, on a wet cloudy day or in the cold atmosphere of the winter even a very strong stimulus evokes only a feeble response, indicating its sluggishness in that environment. Besides environment the excitability has also been found to be related to the age of the leaf. A very young leaf is much less excitable than a fully grown young leaf. The excitability is diminished progressively in older leaves.

According to J. C. Bose the leaf in its excitatory response releases energy, accumulated in its tissue, absorbed from environmental sources. The energy it receives in the form of a stimulus is insignificant in comparison to the amount of energy released in each response. The stimulus only induces a trigger action to bring about the release of the internally held energy.

Though stimulus is an essential prerequisite to provoke a response, occasionally some leaves may be found to exhibit an excitatory fall without any apparent cause of stimulation. J. C. Bose[1] first observed this phenomenon in some plants growing in the open garden. At first he thought that this might be due to the wind current. In order to be definite he enclosed some plants within a glass chamber and observed that even in that guarded condition some leaves, in an autoexcitable manner, might occasionally exhibit such responsive movement. He did not pursue the problem further. He, however, concluded that such response is due to the over-accumulation of energy in the tissue which the leaf releases, in a semi-automatic manner, in a highly excitable condition of the plant. The plant even under normal condition is subjected to the action of various stimuli supplied by its environment. His contention was that it is from the joint action of such stimuli, received from different sources, the tonic condition of the tissue may be raised above the optimum and the excitation thus initiated in the supertonic tissue evokes a seemingly spontaneous response.

Excepting the observation of J. C. Bose no other reference is available regarding this spontaneous activity of the leaf. Hence it may be assumed that this particular phenomenon escaped the notice of other workers. Bose's observation also may be called as casual. He did not attempt to investigate scrutinizingly into the problem with the help of his self-recording devices.

Later on Sri G. C. Bhattacharya, another worker of this Institute, made some confirmatory observation (unpublished) on Bose's observation regarding this spontaneous response of Mimosa leaf. He also observed this casually in the plants growing in the Institute garden. The present authors stumbled into this problem while engaged with the investigation of the diurnal changes of excitability of the leaf. The spontaneous response of the leaf became conspicuous in some of the records. It was then proposed to ascertain the conditions under which such autogenic responses of the leaf might appear.

In order to make a detailed study of the phenomenon arrangements for continuous recording of the diurnal movements of the individual leaves became imperative. Because such records alone could show definitely whether all the leaves occasionally undergo such autogenic response or the phenomenon is limited only to the leaves of some particular age group. The frequency of its occurrence in the same leaf may also be definitely known from such records. Besides, form the time of its occurrence it may be easier to understand if it is influenced in any way by some environmental factors.

METHODS

For taking the diurnal record of the leaf movement a single lever twentyfour-hour automatic recorder, devised by J. C. Bose, was used with slight modification. In these types of recorders the point of the recording lever is not in constant touch with the smoked glass plate on which the record is taken. The smoked glass plate is made to oscillate forward at intervals of fifteen minutes by a clockwork, to take an impression from the point of the lever with which the plant is attached. The impression of the lever point in the form of a dot indicates the position of the lever at the particular time. To record the excitatory impulse of Mimosa the frequency of oscillation of the recording plate has to be much increased so that a response occurring within the long fifteen-minute interval may not be missed. This would necessitate a very big clockwork system. For this reason the intermittent recording system was abandoned and continuous line recording device was substituted. In doing so a thin brass wire was beaten into a delicate flat spring and was attached to the top portion of the glass lever. The curved tip of the spring touches the smoked plate very lightly. The adjustment of the strength of the spring and its pressure on the smoked plate are essential to minimise the frictional resistance at the recording point of the lever. This was accomplished after repeated trials.

In recording the movement of the leaves the plant was enclosed with the recorder in a glass chamber having proper ventilation arrangement. The glass chamber was placed in a south-facing open corridor so that the plant though not exposed to the sun could get sufficient diffused light in the daytime.

The petiole of the leaf was attached to the opposite side of the lever by unspun silk thread. Therefore the down movement of the leaf is represented by the up curve in the records and vice versa.

RESULTS OF THE FIRST SERIES OF EXPERIMENTS

To start with the investigation, diurnal movements of the leaves of varying ages, from very young to full maturity, were individually recorded for several days in succession. The object of such study was to know whether this characteristic responsive movement occurs occasionally in all the leaves or it is restricted to the leaves of any particular age group. Moreover the diurnal records might reveal if this phenomenon is, in any way, associated with the variations of the conditions of the environment, such as light, temperature and humidity.

From a large number of experiments it appeared that this phenomenon is limited to the leaf in its young stage. As the leaf advances to maturity, the frequency of autogenic responses gradually declines, ultimately becoming nil. The first leaf at the top shows the maximum activity in this autogenic behaviour. In the second leaf this activity was found to be greatly diminished. While in the leaves situated further down the stem this activity is totally absent.

Recording of the first leaf was started when its petiole straightened after emergence from the top bud. The pinnules are only half open then, but the leaf attains its full size within two to three days in favourable growing seasons. The leaf in such condition exhibits the activity of autogenic response for about a week. By that time, however, it is no longer the first leaf because the apical bud had brought forth another leaf above it. The frequency of autogenic response in such condition of leaf is maximum within the first two or three days, after which it gradually declines till it becomes nil. A series of diurnal records of such a leaf is presented in Fig. 1.

It will be seen from the records that the maximum number of autogenic responses have occurred on the second day (2). It will be found from counting that the total number of responses were ten in that day's record. This maximum number was quite high for most of the specimens. In a few cases only a higher figure has been obtained.

In this series of records the number of responses has reduced to one on the 6th day, and on the 7th day there was no response. Almost in all cases this autogenic behaviour of the leaf totally disappeared within a week.

It will be observed that sometimes two or three responses have closely followed one another. This phenomenon has been noticed generally to occur in that condition of the leaf, when it executes a considerable number of responses in the twentyfour-hour period. Sometimes the recovery from a response is hardly completed when another response occurs. It indicates that whatever may be the source of excitation its impact may sometimes appear in quick successions.

It will be further noticed that the responses appear to be randomly distributed during the twentyfour-hour period. The occurrence of the responses cannot thus be correlated with any one environmental change like temperature, light or humidity.

In many records a response is found to have occurred during the evening when the leaf was undergoing its characteristic evening movement. This peculiar movement of the leaf in the evening will be dealt with in a separate communication. During the evening with the fall of light and temperature the leaf gradually sags downward and then rises again to its original position covering a period of two to five hours in different specimens. An autogenic response occurring at that period may be considered to be due to the effect of changes in the environmental conditions. But responses occur at other times of day and night as well when no such concomitant changes of any environmental factor are present. From analysis of a large number of diurnal records the responses can hardly be attributed to the diurnal changes of environmental condition.

This does not mean that sudden change of an environmental condition cannot provoke a response. It has been observed that when plants are enclosed in a darkened condition for about two hours and then exposed to normal light all on a sudden, many of the young leaves undergo excitatory responses. Such abrupt change of an environmental factor may be sufficient stimulation for many leaves to execute an excitatory response. In normal change of environment in nature such abruptness is a rare phenomenon.

When no apparent correlation could be found between the responses and the changing environmental factors like light, temperature and humidity, it was assumed that the irregularly occurring responses might be the effect of the random impact of cosmic ray particles on the pulvinus of the responding leaf. To test the validity of such an

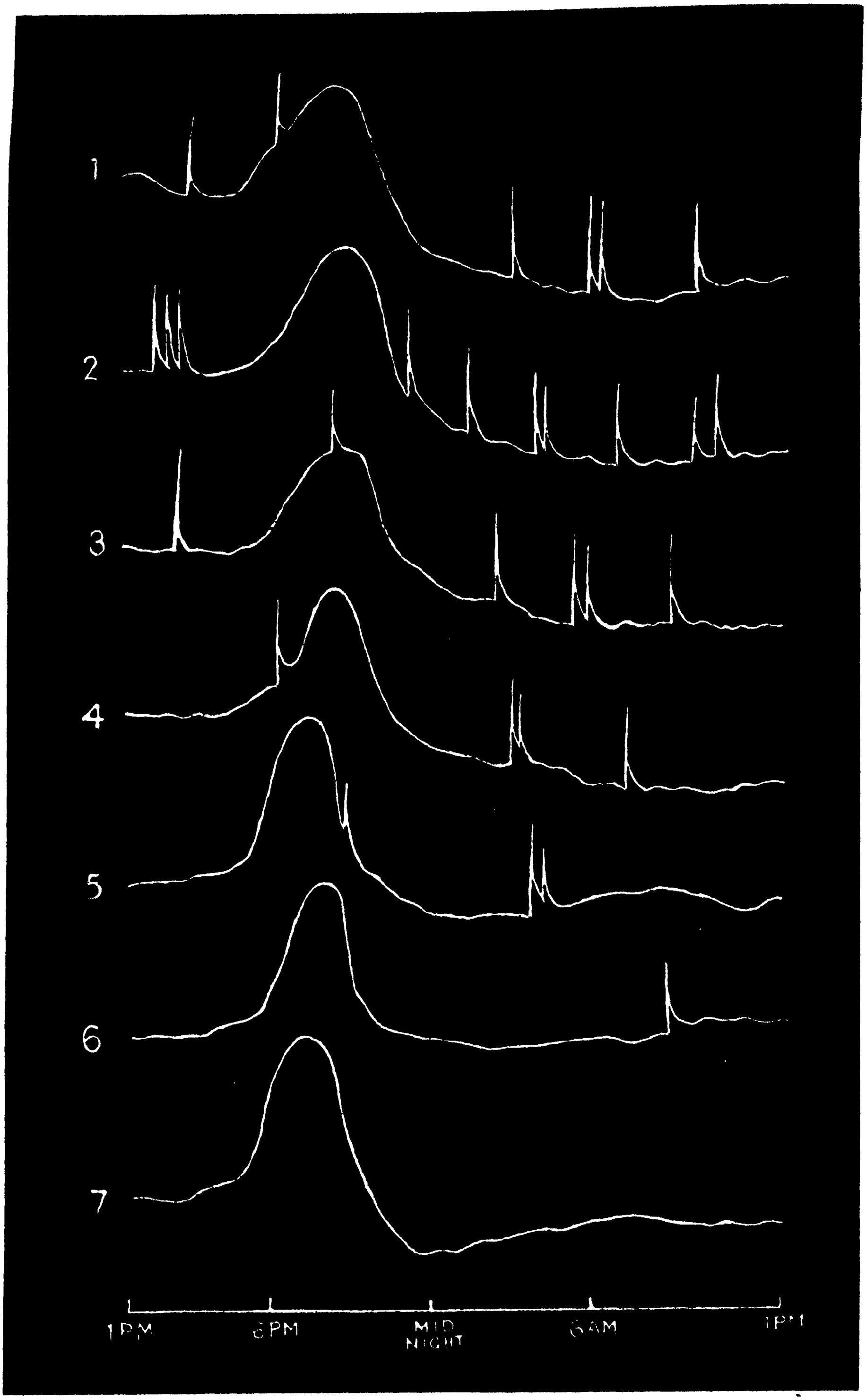


Fig. 1. Diurnal records of the movement of a young leaf of *Mimosa pudica* showing autogenic responses. Records of seven consecutive days starting from very young stage of the leaf are given in the curves 1, 2, 3, 4, 5, 6 and 7.

Note decrease in frequency of autogenic responses in the lower curves with advancement of maturity of the leaf.

assumption, a Strontium 90 source, emitting beta rays (as well as γ-rays), was placed near the pulvinus. This continuous impact of the rays upon the pulvinus and the leaf was not found to change, in any way, its normal behaviour in respect to autogenic response.

RÔLE OF HORMONE IN THE OCCURRENCE OF AUTOGENIC RESPONSE

It has been found that when the top bud is removed the leaf totally loses its autogenic activity. The bud was cut out by a sharp razor blade generally on the second day, after the first day's normal record had been taken. It is shown in Fig. 2, how the autogenic activity of the leaf disappears in the 2nd, 3rd and 4th days' records after the removal of

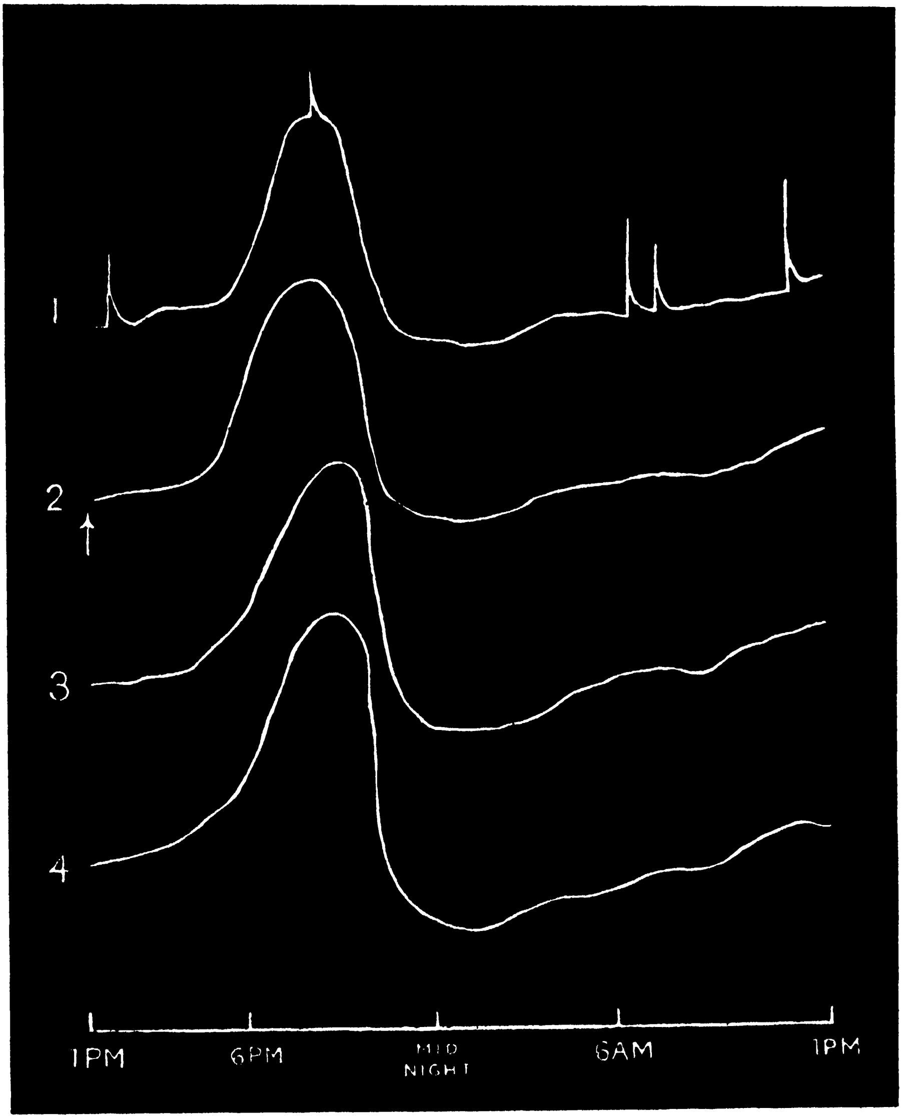


Fig. 2. Showing abolition of autogenic responses after removal of the apical bud. Curve 1, normal record; curves 2–4, under detipped condition. The bud was removed at the beginning of the second curve indicated by the arrow.

the top bud. The removal of the top bud does not bring about any change in the general curve of the diurnal movement of the leaf. This will be quite clear from comparison of the lower three curves with the top one. The normal progress of development of the young top leaf is not found to be affected under debudded condition. This becomes apparent from comparison with similar leaves in other plants.

Since the autogenic responses disappear under debudded condition it may be reasonable to assume that the secretion of hormone from the top bud might have some relation with the occurrence of autogenic responses. On this assumption, experiments were undertaken to observe how the leaf behaves under debudded condition when treated with auxin at the cut surface.

In these experiments also the top bud was removed after the completion of the first day's normal record. After the removal of the bud a preparation of 0·01% indole acetic acid in lanoline paste was applied, in thin film, on the cut surface. The distance between the cut surface and the young leaf being very small, precautions were taken so that its pulvinus did not, in any way, come in direct contact with the auxin during application and afterwards.

Continuous records of a leaf are given in Fig. 3, to show the effect of auxin treatment. The first curve containing seven autogenic responses represents the normal record of the leaf. Debudding and auxin application were made just before the starting of the second day's record. The presence of autogenic responses, though reduced in number, in the 2nd to 4th days' records, indicates that the influence of debudding has at least been partially removed by the application of auxin. The results of a series of experiments showing the effect of debudding and that of auxin application at the cut stump are given in Table I.

TABLE I

Showing abolition of autogenic responses in diurnal records under detipped condition and partial retention of the same by treatment with 0·01% I.A. in lanoline

Sp. No.	No. of autogenic responses	No. of autogenic responses in detipped condition				
	1st day	2nd day	3rd day	4th day	5th day	6th day
1	5	Nil	Nil	Nil		
2	6	Nil	Nil	Nil		
3	3	Nil	Nil	Nil		
4	7	Nil	Nil	Nil		
5	6	Nil	Nil	Nil		
6	4	Nil	Nil	Nil		
7	7	Nil	Nil	Nil		
		No. of autogenic responses with application of 0·01% I.A. after decapitation				
1	7	3	2	3	1	1
2	4	2	2	x	x	x
3	8	1	4	2	Nil	
4	6	2	2	1	1	
5	9	3	1	Nil	x	
6	5	3	3	2		
7	4	Nil	3	1	2	Nil
8	6	2	3	1	x	x

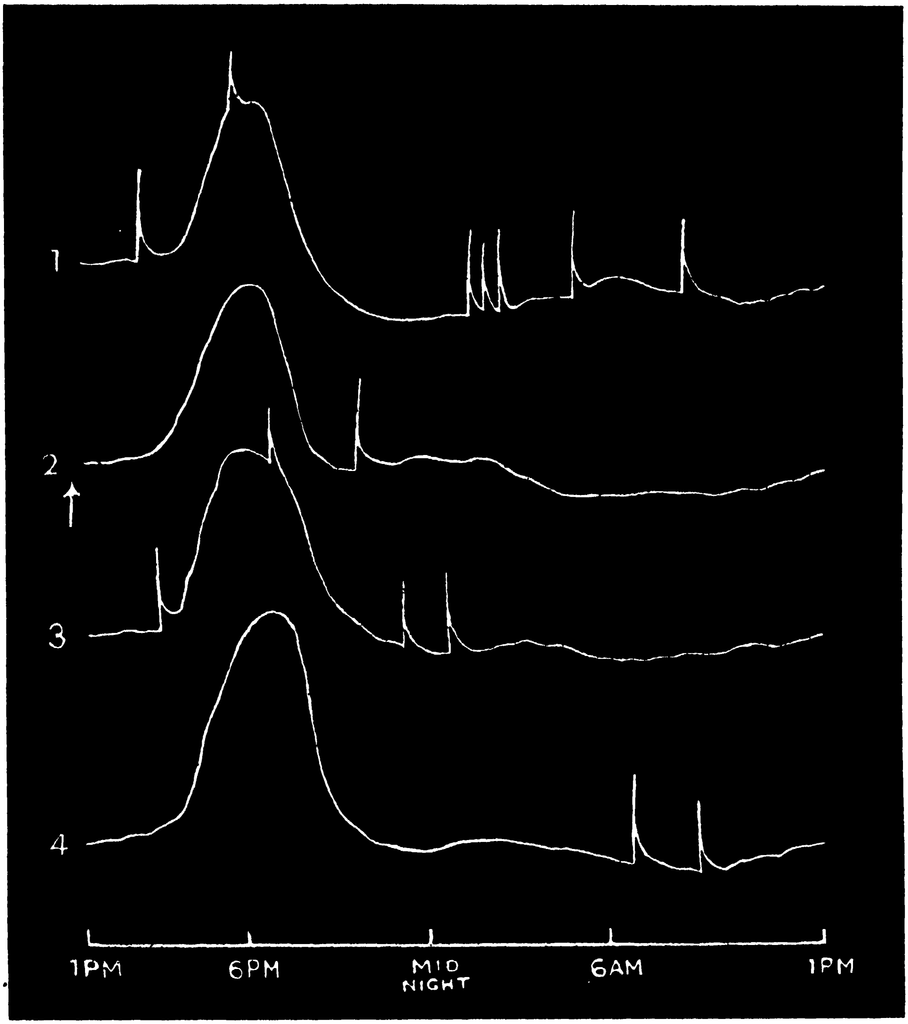


FIG. 3. Showing partial revival of autogenic responses by application of 0·01% I.A. on the cut stump. Curve 1, normal record; curves 2–4, under I.A. treatment after the removal of the tip at the arrow.

DIURNAL VARIATION OF FREQUENCY DEDUCED FROM A LARGE NUMBER OF RECORDS

From general examination of a large number of records it becomes apparent that though the responses can occur randomly at any part of day and night there is some variation in their distribution during the twentyfour-hour period when all the records are taken into consideration. More responses seem to have occurred in some periods than in others. It was then proposed to work out the frequency of response in relation to individual hours of the day, from data computed of a large number of records. For computing the data those records were included which contained at least four responses in them. Fifty such records were taken for the present assessment. All the responses in these fifty records were individually grouped out on hourly basis. The total number of responses thus obtained at different hours of the day is graphically represented in Fig. 4. The frequency curve indicates two distinct peaks, one between 2 and 4 p.m. and the other between 9 and 11 a.m. The curve also indicates that there is tendency of higher frequency during the day than at night.

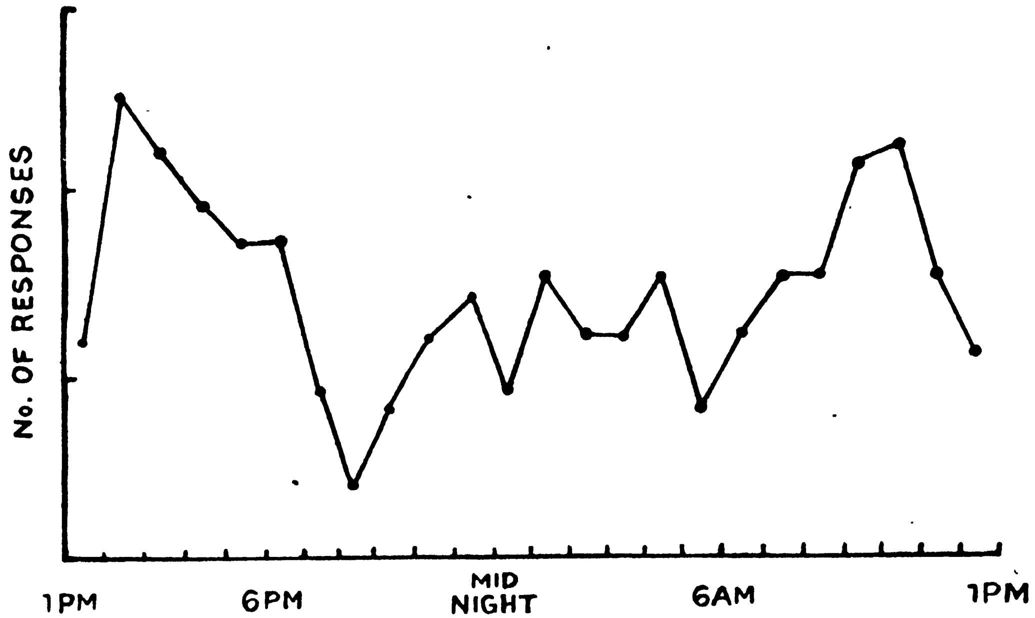


FIG. 4. Frequency of autogenic responses in *Mimosa* during twentyfour-hour period computed from fifty diurnal records.

DISCUSSION

It will be evident from the records that the leaf of Mimosa can often undergo a responsive movement without any apparent cause, quite similarly as it normally does under a stimulation. J. C. Bose who first observed the phenomenon concluded that such responses can occur in the highly excitable condition of the leaf. According to him when the leaf attains a supertonic condition by the accumulation of excess amount of energy from different environmental sources, it performs a response without any immediate cause of external stimulation, to be relieved of that energy. Bose's experiments have shown that the leaf is more excitable during the day than at night. The tonic level is, no doubt, raised higher at day by the absorption of light and higher temperature. Bose's observation being limited to the daytime it was quite plausible for him to reach such a conclusion. But the diurnal records presented in this paper clearly show that these responses do not occur during the day alone; they occur at night as well. The occurrence of the response has no time restriction; they may be performed quite randomly at all periods of day and night. It, therefore, seems that the performance of the response is not dependent on the higher excitability phenomenon of the leaf.

Moreover, the present investigation reveals that the leaf exhibits this responsive phenomenon at its young stage only, completely disappearing with the maturity of the leaf. Bose has shown that the young leaves are less excitable than the mature ones. This further shows that the phenomenon is not related with the higher excitability condition of the leaf.

Diurnal changes of environmental factors like temperature, light and humidity individually do not seem to be the causative factor for the occurrence of the response. The failure of the exposure to beta rays from close range to induce any change on the normal behaviour of the leaf, in respect to the autogenic response, sufficiently indicated that the random impact of cosmic ray particles has no relation with such responses. It may, therefore, be reasonably assumed that the cause of response does not lie in the stimulatory action of any external agency. In accepting this assumption our attention becomes obviously directed toward the internal changes occurring in the plant tissue. But whatever may be this internal cause, it can be said that the raised tonic level of the tissue is not directly related to it.

Cessation of autogenic activity under detipped condition and its partial revival by the application of auxin on the cut surface at the tip clearly indicates that the hormone mobilisation from the apical bud has some influence on the occurrence of the autogenic response of the leaf. But how it reacts to bring about the activity is not clear. Moreover the hormone is also mobilised to other leaves down the stem; but it seems to fail to bring about similar reaction there. The young top leaf due to its close proximity to the apical bud, no doubt, receives greater supply of hormone to be utilised there in its rapid developmental processes.

In a previous communication[2] on the periodicity of growth it has been shown how the periodic acceleration of internodal growth in *Cinchona ledgeriana* appears to be related with the release of hormone during the bursting of the apical bud. In that case also the effect of hormone in inducing such acceleration was found to be more pronounced in the apical region. It is quite conceivable that the apical region, by virtue of its position, receives greater supply of hormone and its requirement there is also greater than elsewhere due to high meristematic activity. In the present case, though apparently the normal development of the young leaf is not much affected by the removal of the apical bud, yet the hormone may be associated with some reaction, within the tissue, in the developmental process, which acts as a stimulation to the leaf causing a response. If such an internal reaction is associated with the responsive phenomenon, it seems to have no regularity in its occurrence.

Again though the response can occur at any hour of the day, the frequency curve indicates that there are two peak periods in it yielding the highest number of responses. Both of these periods are in the daytime, one between 9 and 11 a.m. and the other between 2 and 4 p.m. It may be difficult to guess how these particular periods are more favourable for the expression of the autogenic activity. In considering the curve as a whole it may be said that the activity is comparatively lower during the night than in the daytime. Though the occurrence of the response cannot be correlated with the change of any one of the environmental factors, the light and temperature may have some relation with the enhanced activity during the day. If the autogenic response is brought about as a result of some reaction produced by the hormone, the intensity of that reaction is likely to vary by the influence of such environmental factors.

Besides, light is closely related with the production of hormone in the plant. Yin[3] in working out the periodic concentration of auxin of different regions of the leaf and petiole of *C. papaya* leaf, in connection with the study of its nyctinastic behaviour, obtained two maxima in the periodic curves, one in the afternoon and the other in the morning. He presumes that the maximal concentration in the afternoon is due to active production of auxin in light and the maximal concentration in the morning as being due to the transformation of auxin precursor to auxin. In the pulvinus of Mimosa also the possibility of such periodic variation in the concentration of its hormone content is not excluded. Hormone is related with irreversible growth changes in papaya leaf and this function of hormone universally applies to all plants. In the leaf of Mimosa the autogenic activity also seems to be dependent on hormone, though it is not known how it reacts to induce the movement. High frequency of response in the very young stage of the leaf is quite indicative that high concentration of hormone stimulates the activity. It can, therefore, be presumed that the peak periods of autogenic activity may have relation with the periodically increased concentration of hormone in the pulvinus.

SUMMARY

Mimosa leaf has been found occasionally to exhibit a responsive movement without any apparent cause.

Continuous diurnal record of the individual leaves reveals that such autogenic responsive movement is present in the leaf only in its young stage.

The frequency of such movement is highest when the leaf is very young and it is gradually reduced with advancement in maturity.

The responses occur quite randomly at different periods of day and night and they cannot be correlated with the changes of environmental factors like light, temperature and humidity.

It has been found from experiments that the random fall out of cosmic ray particles are in no way correlated with the irregularly occurring responses of the leaf.

Removal of the apical bud stops the autogenic activity of the young leaf, which is partially revived by the application of 0·01% indole acetic acid in lanoline paste on the cut stump.

The frequency curve computed from fifty records indicates comparatively higher frequency in the daytime.

It has been assumed that the hormone translocation from the apical bud may be associated with some internal reaction in the developmental phase of the young leaf, which acts as a stimulus to induce the response. The comparatively higher frequency during daytime may be due to the influence of light and higher temperature on the internal reaction.

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XV. X-RAY INDUCED MUTANTS OF ECONOMIC IMPORTANCE IN TIL (*SESAMUM ORIENTALE* L.)

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1. INTRODUCTION

Muller (1927) working on Drosophila discovered that X-rays can induce chromosome aberration leading to genetic variability. But many of the subsequent workers in this line, especially in plants, opined that this technique did not have much practical application in plant breeding, since most of the mutants obtained were not useful or better than their controls. The practical utility of this method in plant breeding was first demonstrated by Gustafsson and his collaborators in 1935, and since then they have obtained vital and other mutants with increased yield, disease resistance and other favourable agricultural characteristics in barley, wheat, oats, flax, soybeans, sweet lupins, oliferus species of cruciferae, etc. In U.S.A. also, this method is being successfully used at present in various crop plants, in which higher yielding and disease resistant strains have been obtained in a comparatively short period (Jacob, 1956).

The principal objects of breeding work on any oilseed crop are the improvements both in the yield of seeds per acre and quality and quantity of oil. Since the oil content can be assessed only after harvesting, it is difficult for plant breeders to select higher oil yielding types in the field itself. Again, the determination of the oil content is a lengthy and laborious process, for which the breeder has to depend upon the analytical chemist. Consequently, this character cannot be used as one of the basis in the earlier stages of selection in the field, although it is the most important factor. It is therefore advantageous, if possible, to correlate this character of higher oil content with some other visible characters of the seeds, such as seed size and colour, weight, maturity, etc., and other characters like pigmentation of plant parts, root habit, etc. This may be possible in induced mutagenesis, where very wide genetic variability can be induced, so that some of the single plants selected may have the desired combination of economic characters.

2. EXPERIMENTAL RESULTS

During the course of our experiments with X-rays, two recessive morphological mutants have been obtained, one of which had fully white petals (hereafter referred to as the 'white flowered mutant', as against the purple pigmented petal of the control). This was isolated in the X_8 generation of 1952, from the 20,000 *r* treatment on the dry seeds of *S. orientale* type 16, from West Bengal.

The other mutant, also from the same type, had smaller seeds than the control and was isolated in 1954, from the 14,400 *r* treatment (hereafter referred to as the 'small seeded mutant'). These have been briefly reported earlier (Rai and Jacob, 1956).

The present paper therefore gives in greater detail their performances regarding the yield, chemical properties and peroxide values of their oils and the duration of flowering.

3. OBSERVATIONS

(1) *Yield of oil*

Table I gives the statistical analysis of the oil in the X_5 generation in the white flowered and small seeded mutants, as against their control.

TABLE I

Mutants and control	Mean per cent ± S.E.	Mean per cent ± S.E. over control
ntrol	41·49 ± 0·51	100·00 ± 1·23
ite flowered mutant ..	44·06 ± 0·51	106·18 ± 1·23
all seeded mutant ..	52·10 ± 0·51	125·56 ± 1·23

It will be seen from Table I that the white flowered mutant gives nearly 6% and the small seeded mutant nearly 26% more yield of oil over the control.

(2) *Chemical properties of oil*

Chemical properties of the oils of the white flowered and small seeded mutants have been considered under the following heads.

(*a*) *Colour.* This is an important criterion in edible oils. To the general consumer, the lightness of colour is a pointer to the degree of purity and freshness. The colour was evaluated by a Lovibond Tintometer and the results were reported in yellow and red units of Priest-Gibson colour scale (Gibson and Harris, 1927; Gibson and Haupt, 1934; McNicholas, 1935).

(*b*) *Refractive index* of an oil mostly reflects its average unsaturation, although its magnitude is governed also by all the major and minor constituents of the oil. Roughly speaking, the higher the refractive index at a particular temperature, the higher the unsaturation.

(*c*) *Saponification value* indicates the average molecular weight of the fatty acids, while the *iodine value* gives the average unsaturation.

(*d*) *Non-saponifiable* matters are minor constituents, e.g. colouring matters like carotenes, anthocyanins, etc., steroid materials, vitamins and few others which are not saponifiable and are expressed as percentage non-sap (% NS). Their properties influence the keeping quality of oils and also determine the suitability of the oil for edible purposes, e.g. the presence of the toxic substance, gossypol in cotton seed oil prevents its use as an edible oil. Sesamol, Sesamoline and Sesamin that are present in the NS of sesamum oil are harmless and do not possess toxicity. On the contrary, they hinder the process of auto-oxidation of the oil and allow the oil to keep for a long time (Olcott and Matill, 1941; Budowski, 1950; Moore and Bickford, 1952).

(*e*) The properties of the triglycerides of vegetable oils depend upon different fatty acids that are present in the form of glycerol esters and also upon the arrangement of the fatty acids in the triglyceride molecule.

Table II shows the chemical properties of the oil of the white flowered and small seeded mutants and their control type 16.

It will be seen from Table II that there are no significant differences in the chemical properties of the oil of the white flowered and small seeded mutant and their control.

TABLE II

Mutant and control	Characteristics of oil					Composition of mixed fatty acids		
	Colour by Lovibond scale	Refractive Index ND 20°C.	Saponification value	Iodine value	% Non-sap	Saturated acids	% Oleic acids	% Linoleic acids
Control (type 16) ..	Y = 1·6 R = 0·4	1·4737	192·2	108·0	1·66	14·1	44·5	41·4
White flowered mutant ..	Y = 1·5 R = 0·3	1·4738	192·0	109·2	1·92	14·3	47·4	38·3
Small seeded mutant ..	Y = 1·5 R = 0·3	1·4733	192·4	102·0	1·68	15·0	45·5	39 5

(3) *Peroxide value*

The degree of rancidification of a sample of oil can be ascertained from its peroxide value. It is expressed in terms of c.c. of 0·0002 N thiosulphate required to titrate the liberated iodine, when 1 gm. of fat is treated with powdered potassium iodide in a mixture of glacial acetic acid and carbon tetrachloride or chloroform. This was determined by the simplified method of Lea (1931).

Table III shows the peroxide value of the oil of the white flowered and small seeded mutants and the control.

TABLE III

Mutant and control	Freshly extracted oil	Oil kept for a month
Control (type 16) ..	6·6	21·5
White flowered mutant ..	6·4	10·4
Small seeded mutant ..	2·2	9·5

The freshly extracted oil of the white flowered mutant does not show marked differences in its peroxide value from the control which is 6·4 and 6·6 respectively. But when the oils were kept for one month and then analysed, it was found that the peroxide value of the mutant was 10·4 as against the control having 21·5. But the oil of the small seeded mutant with a peroxide value 2·2 has much better keeping quality than that of the control with peroxide value 6·6. After a month, although there is an increase in the peroxide value of these oils, the oil of the small seeded mutant with its peroxide value 9·5, show better keeping quality than the oil of the control with its peroxide value of 21·5. Therefore, it appears that the oils of the mutants have better keeping qualities.

(4) *Flowering behaviour*

Table IV shows the flowering behaviour of the white flowered and the small seeded mutants as against their control type 16.

TABLE IV

Mutant and control	Date of sowing	Date on which first plant flowered	Age at the time of flowering in days	Earliness and lateness	Date on which last plant flowered	No. of days covered	Date on which maximum number of plants flowered
Control ..	28-3-57	6-5-57	40	..	20-5-57	15	9-5-57
White flowered	,,	10-5-57	44	4 days late	24-5-57	15	16-5-57
Small seeded mutant ..	,,	28-4-57	32	8 days early	21-5-57	24	12-5-57

It will be seen from Table IV that a selection in the small seeded mutant flowered 8 days earlier than the first flowering plant of the control, whereas in the white flowered mutant, the first flowering selection was 4 days later than the control. Though the duration of flowering in the control and the white flowered mutant is the same, i.e. 15 days, in the small seeded mutant it is 24 days. The date on which the maximum number of plants flowered both in the white flowered and the small seeded mutants are 16-5-57 and 12-5-57 respectively, as against 9-5-57 of control, i.e. they are 7 and 3 days later respectively than the control.

When the data was statistically analysed (Table V) the white flowered mutant proved to be later by 3·4±0·36 days, while the small seeded mutant was earlier by 1·2±0·33 days, than the controls.

TABLE V

Mean time of flowering with standard error

Mutant and control	Mean (in days) ± S.E.	No. of plants observed	Difference ± S.E.
Control	45·9 ± 0·19	338	..
White flowered mutant ..	49·3 ± 0·30	132	+ 3·4* ± 0·36
Small seeded mutant ..	44·7 ± 0·27	170	− 1·2* ± 0·33

*Denote 1 % level of significance.

4. DISCUSSION AND CONCLUSIONS

It is now known that the carbohydrate, protein, fat and vitamin content of corn endosperm can be changed to a certain extent by controlled breeding. Various opinions have been expressed from time to time regarding the mutation process as a chemical one involving the chromosomes (Goldschmidt, 1951 ; Sturtevent, 1951). Mangelsdorf (1947) and Cameron (1947) have drawn to some extent the parallelism between genes and carbohydrates in corn. They have shown that besides changing the carbohydrate content, these genes modify the physical appearance of the kernels and the concentration of other chemical compounds. How the morphological effect of the genes, which is presupposed by the physiological effect, brings about an increase in the nicotinic acid has been shown by Teas (1952). The two morphological mutants, white flowered and small seeded, isolated from the irradiated progenies, in Sesamum type 16, described above, show about 6% and 26% increase of oil respectively over their control (vide Table I). The oils of these induced mutants show better keeping quality also, as against their parent type 16 (vide Table III), without undergoing any marked deviation in their chemical properties (vide Table II). Andersson isolated a mutant strain 'primex' in white mustard (Jacob, 1956) which gives an increase of 6% in the oil content. It is also known that the small seeded castor gives greater oil percentage than the big seeded ones (Sethi, 1931). Thus, while the increased oil content appears to be correlated with the two morphological characters, white flowers and small seeds, the earliness in flowering has been found only in the progenies of small seeds (vide Table V). Selection for such combination of characters, earliness and increased oil content is only possible when the genetic variability produced is of a very high order and which is very common in irradiation experiments. Such earliness together with the increased yield of seeds have been obtained in our irradiation experiments with mustard also (vide Table XXXII of Progress Annual Report, 1956-57, submitted to the Indian Central Oil-seeds Committee).

Thus, the two morphological mutants, white flowered and small seeded, with significant increase in the percentage of oil and lower peroxide value, clearly indicate the beneficial effects of the X-rays in inducing the morphological mutants of economic value.

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